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ADENOMATOUS HYPERPLASIA OF THE ADRENAL CORTEX ASSOCIATED WITH ESSENTIAL HYPERTENSION

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For several years we have been impressed with the frequent occurrence of hyperplasia of the adrenal cortex in association with essential hypertension. Recently, a detailed anatomic study has been made of the adrenal glands in cases of hypertension and in cases in which hypertension was not present, in an attempt to evaluate critically this impression.

METHOD OF STUDY

Adrenal glands were removed routinely at necropsies and, after rough dissection of surrounding fat, were fixed in dilute solution of formaldehyde U. S. P. (1:10). Following fixation, the glands were carefully dissected and weighed. Sections were made from blocks taken through the greatest diameter of each gland from apex to base and stained with hematoxylin and eosin.

In classifying the cases, the usual criteria for the establishment of the diagnosis of essential hypertension were used. Instances of significant cardiac hypertrophy without primary renal disease or disease of the cardiac valves or great vessels were tentatively considered to be cases of essential hypertension. In nearly all cases so classified clinical records were available which showed elevated blood pressure. Sections of kidney were examined to eliminate primary renal disease and to study the vascular lesions present. Cases were classified as instances of essential hypertension only if all the data at hand warranted that diagnosis. To avoid confusing data, doubtful cases were not classed as instances of essential hypertension, and it is probable that a number of instances of mild hypertension have been omitted. Cases of cardiac hypertrophy which might have been due to hypertension secondary to an obstruction of the lower part of the urinary tract or unilateral renal disease were not included. Likewise cases of mild cardiac hypertrophy with advanced coronary disease were not included unless there was a clinical record of definite hypertension. Cases in which the adrenal glands were

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involved by metastatic neoplasms, hemorrhage or extensive edema were eliminated. There remained 26 cases classed as instances of essential hypertension. The heart weights in this group ranged from 410 to 1,000 Gm. As control material we had 100 cases in which the heart weights were under 350 Gm. and in which it could safely be assumed that significant hypertension had not been present. In 18 other cases cardiac hypertrophy was due to valvular or chronic pulmonary disease. There were 13 cases of cardiac hypertrophy associated with primary renal disease, 4 with chronic glomerular nephritis and 9 with chronic pyelonephritis.

ANALYSIS OF DATA

An analysis of the material is shown in the table. The mean combined weight of the adrenal glands in the 100 cases of nonhypertensive conditions was 11.2 Gm. ($\pm$ 3.2); the mean combined weight in the cases of essential hypertension was 15.4 Gm. ($\pm$ 3.4). The weight was over 15.0 Gm. in only 8 per cent of the cases of nonhypertensive conditions, as compared with 53 per cent of the cases of essential

Combined Weights of Adrenal Glands

	Cases	Mean Weight, Gm.	Standard Deviation	Cases in Which Combined Weight Was	
				Over 15 Gm.	Under 11 Gm.
Control group	100	11.2	3.2	8 (8%)	53 (53%)
Cardiac hypertrophy and valvular or pulmonary disease	18	10.6	...	0	10
Glomerular nephritis	4	13.1	...	1	1
Chronic pyelonephritis	9	13.3	...	2	2
Essential hypertension	26	15.4	3.4	14 (53%)	5 (19%)

hypertension; in 7 instances in the latter group the glands weighed from 18 to 22.5 Gm. The glands weighed 11.0 Gm. or less in 53 per cent of the cases of nonhypertensive conditions, compared with 19 per cent of the cases of essential hypertension. The adrenal differences in the two groups are even greater than is revealed by gross weight. The large adrenals in the control series did not show the same gross or microscopic picture seen in the glands of the essential hypertension series. The majority of the control glands showed more or less interstitial edema, while those in the essential hypertension group presented a solid cellular cortical substance.

In most cases of essential hypertension the structure of the adrenal glands is greatly altered. The cortex shows a change that is best described as adenomatous hyperplasia. It is irregularly thickened and nodular, as shown in figures 1 and 2. Microscopically, the orderly zonal arrangement of the cells is absent. Cell columns are curved or tortuous, and individual cortical cells are larger than normal (fig. 3). Many of the cell nuclei are large and hyperchromatic, and in nearly all of the glands there are groups of cells in which the cytoplasm is stuffed with

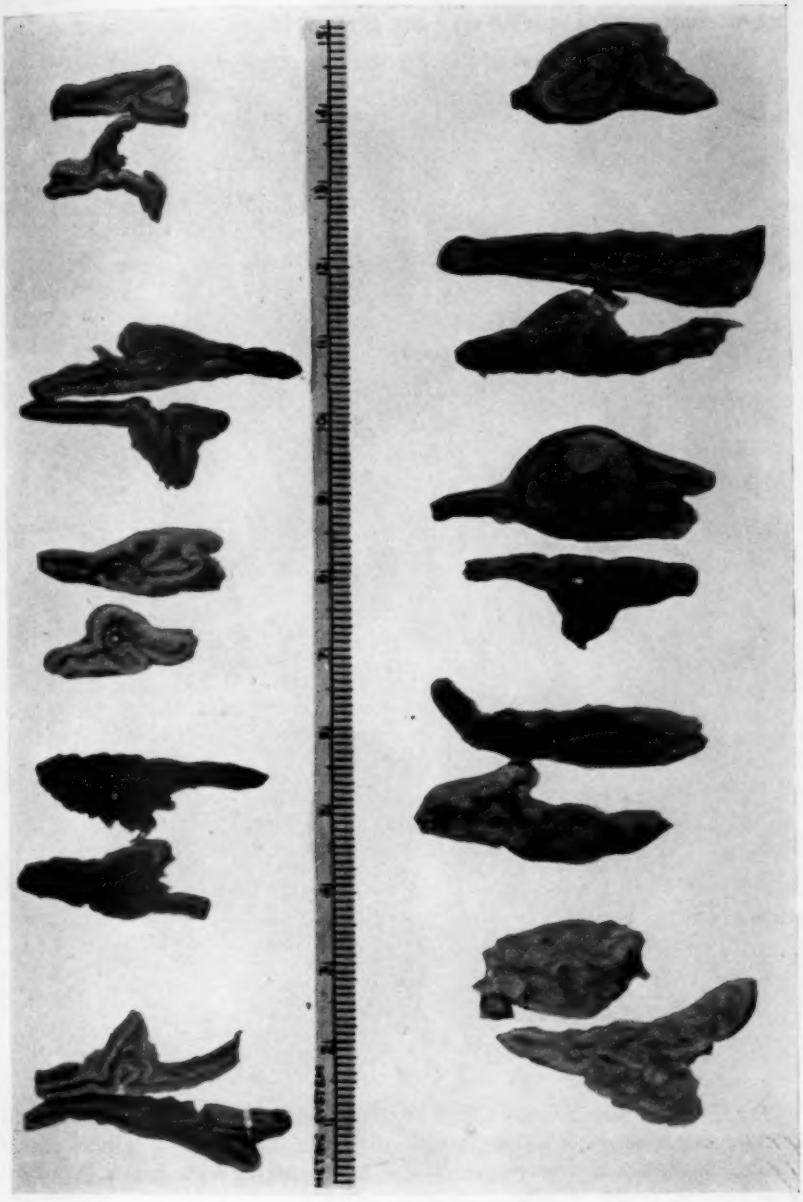

Fig. 1.—Left row shows adrenal glands of average normal size and contour obtained at necropsies in cases of nonhypertensive diseases. The right row shows adrenal glands obtained in cases of essential hypertension. The larger size and irregular nodular thickening of the cortex are evident.

fine lipoid droplets (fig. 3 *B*). The changes described are well defined in the adrenal glands in 20 of the 26 cases of essential hypertension, and lesser changes of a similar type are present in the remainder. Zwemer[1]

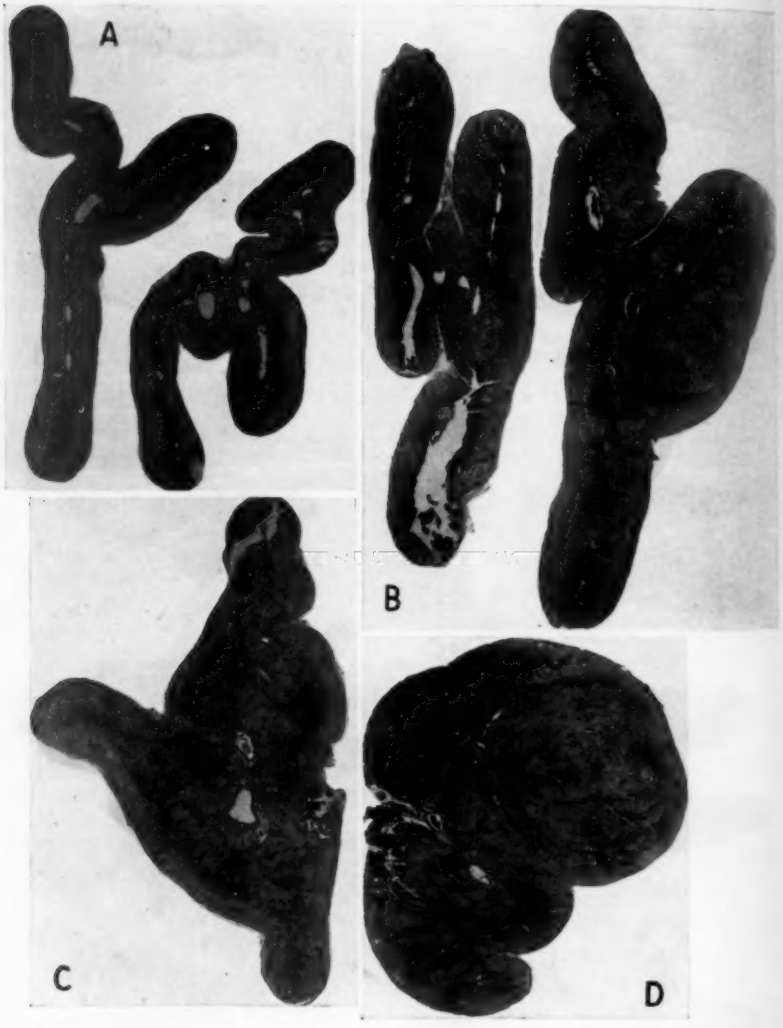

Fig. 2.—Low magnification (× 3) of sections of adrenal glands, illustrating the adenomatous hyperplasia of the adrenal cortex found in cases of essential hypertension. *A*, glands of average normal size from a person with chronic glomerulonephritis. *B*, *C* and *D*, adrenal glands from patients with essential hypertension.

has associated a high lipoid content in the adrenal cortex with a stimulated gland. Ten of the 100 control glands showed significant degrees

1. Zwemer, R. L.: Am. J. Path. **12**:107, 1936.

of adenomatous hyperplasia, and in 7 the microscopic changes were similar to those of essential hypertension, although the appearance of active hyperplasia was not as striking, and it is noteworthy that the glands

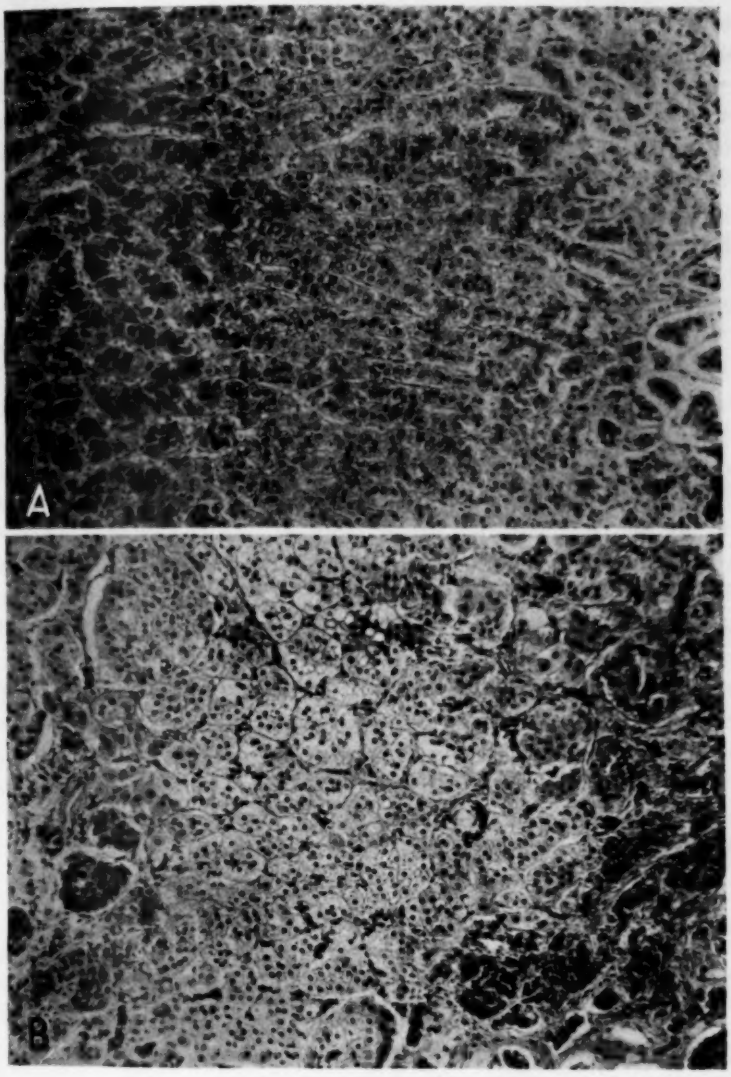

Fig. 3.—*A*, normal cortex showing regular zonal arrangement; × 75. *B*, portion of cortex in a case of essential hypertension, showing irregular cell columns. The cells are large, and many show a high lipoid content; × 75.

in the latter control group were not large. The average combined weight was 12.5 Gm., and none weighed over 13.5 Gm. (In 4 of the 7 cases the glands were from patients with severe infection.) Most of the

enlarged glands in the control series showed a diffuse, usually regular thickening of the cortex, and the cells contained lipoid in normal or, more commonly, diminished amounts. In 5 of the cases of hypertension, one gland showed a more or less well circumscribed nodule, approximately 1 cm. in diameter, that might be classified as adenoma. It is noteworthy that, except in 1 instance, this occurred in conjunction with adenomatous hyperplasia of the remainder of the cortex. In 1 case a sharply circumscribed tumor diagnosed as adenoma was centrally located in one gland (fig. 1). In 3 instances the control glands showed comparable tumors classified as adenoma, one accompanied by adenomatous hyperplasia of the remainder of the cortex. The adrenal glands in the 18 cases of cardiac hypertrophy due to valvular or pulmonary disease did not differ in size or microscopic appearance from those in the control group.

The 13 cases in which cardiac hypertrophy was associated with primary renal disease constitute an interesting group. In 4 cases, in which the renal condition was glomerular nephritis, the adrenal glands weighed 10.8, 12.3, 13.0 and 16.6 Gm. In 1 of these 4 cases there was mild adenomatous hyperplasia of the cortex and increased lipoid. This occurred in a man aged 73 in whom preexisting essential hypertension could not be excluded. Arteriolar sclerosis was particularly marked in this case. In the remaining 3 cases the adrenal glands appeared essentially normal. In 9 cases, in which the renal disease was chronic pyelonephritis, the weights of the adrenal glands ranged from 9.6 to 16.6 Gm., with an average combined weight of 13.3 Gm. In 2 of these cases there was well defined adenomatous hyperplasia of the cortex, comparable to that seen in essential hypertension; in 6 others there was very mild hyperplasia or slight cortical thickening; in 8 more than the usual amount of lipoid was present in the cortical cells and these appeared functionally active. This suggests that there is some stimulation of the adrenal cortex in renal hypertension.

COMMENT

The significance of this cortical hyperplasia which we have shown to be commonly associated with essential hypertension offers a problem not readily solved. It is not related to age or body weight. Control cases in which the age range of the patients was similar showed a lower mean weight of the adrenal glands than the general average given in the table and cases in which the range of body weights was comparable did not show any difference from this average. Likewise, arteriosclerosis is not a factor. The adenomatous hyperplasia does not occur with arteriosclerosis in the absence of hypertension. The enlargement of the adrenal glands is clearly due to an irregular adenomatous hyperplasia of the

adrenal cortex. No attempt has been made to determine the amount of medullary tissue present, though it appears less prominent than in the control glands. This is probably due to displacement associated with the irregular cortical thickening.

The studies of Prinzmetal and Wilson [2] indicate that there is a circulatory pressor substance in essential hypertension which acts directly on arterioles and is not mediated by the nervous system. The well known work of Goldblatt and co-workers [3] showing hypertension to result from constriction of renal arteries has directed attention to the kidneys as a source of this substance. However, if this mechanism is operative in clinical essential hypertension, either a structural change must be present in the renal arteries or arterioles or one must postulate some extrarenal mechanism to initiate the vasoconstriction. It is possible that a substance from the adrenal cortex may act in this manner.

There is considerable evidence implicating an adrenal abnormality in essential hypertension. Most interest has been centered on the adrenal medulla,[4] but aside from the well established syndrome of paroxysmal hypertension in cases of pheochromocytoma there is little evidence to support the hypothesis of hyperepinephrinemia in hypertension.[5] There are, however, a number of cases reported of hypertension associated with tumor of the adrenal cortex. Oppenheimer and Fishberg [6] collected from the literature 9 cases of neoplasm of the adrenal cortex associated with clinical hypertension or with otherwise unexplained cardiac enlargement and added 2 cases of their own. One of their cases and several of the others fell into the well recognized category of adrenal virilism. Of particular interest, as noted by Oppenheimer and Fishberg, were the 2 cases observed by Volhard [7] presenting the clinical picture of diffuse nephritis with hypertension and albuminuria in which the hypertension disappeared in each instance following the removal of a tumor described as hypernephroma. Pertinent also are the reports [8] of the common finding of hyperplasia and tumors of the adrenal cortex in the syndrome

2. Prinzmetal, M., and Wilson, C.: J. Clin. Investigation **15**:63, 1936.

3. Goldblatt, H.; Lynch, J.; Hanzel, R. F., and Summerville, W. W.: Am. J. Path. **9**:942, 1933.

4. DeCourcy, J. L.; DeCourcy, C., and Thuss, O.: J. A. M. A. **102**:1118, 1934.

5. Grollman, A.; Harrison, T. R., and Williams, J. R., Jr., in Blood, Heart and Circulation, Publication 13, American Association for the Advancement of Science, 1940, p. 274.

6. Oppenheimer, B. S., and Fishberg, A. M.: Arch. Int. Med. **34**:631, 1924.

7. Volhard, F., in von Bergmann, G., and Staehlin, R.: Handbuch der inneren Medizin, Berlin, Julius Springer, 1931, vol. 6, pt. 1, p. 388.

8. Oppenheimer, B. S., and Silver, S.: Tr. A. Am. Physicians **52**:146, 1937. Kepler, E. J.; Kennedy, R. L. J.; Davis, A. C., and Walters, W.: Proc. Staff Meet., Mayo Clin. **9**:169, 1934.

known as Cushing's disease. Early reports in the French literature are of considerable interest. Vaquez [9] recorded a case of chronic nephritis with hypertension in which a large tumor of the adrenal cortex was found at necropsy and diagnosed as adenoma. This stimulated an investigation of the problem by his junior colleagues, Aubertin and Ambard.[10] They examined the adrenal glands in 8 cases of "interstitial nephritis," in which the blood pressure had been increased and found hyperplasia or adenoma of the cortex in 7 instances; in 3 of these there were large cortical tumors ranging from 4 to 8 cm. in diameter, diagnosed as adenoma. In 19 cases of other conditions, including cases of nephritis without hypertension, a similar condition was observed but once. Their description of the gross and microscopic changes in the adrenal cortex is practically identical with that we have given. Although it seems probable that Aubertin and Ambard were dealing with cases of nephrosclerosis, it is not possible to be sure of this. Oppenheimer and Fishberg noted that in a series of 35 cases of hypertension one of them found cortical adenoma in 5 instances while in 50 cases in which no hypertension was present adenoma was found only once. Allen [11] reported hypertrophy of the adrenal veins in essential hypertension. Clinical data indicating the common "sthenic" habitus of patients with essential hypertension are also in accord with an endocrine influence. Schroeder and Steele [12] on clinical grounds have considered that in a portion of the cases essential hypertension has an endocrine basis. Fishberg [13] has been impressed with clinical and pathologic evidence of the type cited, suggesting that hyperactivity of the adrenal cortex may be concerned in the genesis of essential hypertension. Weiss [14] stated that "increasing numbers of cases have been observed in recent years in which hypertension has been observed in cortical (and medullary) tumors of the adrenals." He noted further: "In a study of the nature of hypertension in toxemia of pregnancy now in progress, we have been impressed by the fact that in the majority of instances previous organic disease of the kidneys has not been present and the absence of the usual mechanisms of hypertension raises the suspicion that hormones are the factors chiefly responsible." The blood pressure reached mild hypertensive levels in several patients with Addison's disease while treatment with desoxycorticosterone was being carried on in the series reported by Ferrebee,

9. Vaquez, H.: Tribune méd. **2**:85, 1904.

10. Aubertin, C., and Ambard, L.: Tribune méd. **2**:119, 1904.

11. Allen, E. V.: Ann. Int. Med. **3**:153, 1929.

12. Schroeder, H. A., and Steele, J. M.: Arch. Int. Med. **64**:927, 1939.

13. Fishberg, A. M.: Hypertension and Nephritis, Philadelphia, Lea & Febiger, 1939.

14. Weiss, S., in Blood, Heart and Circulation, Publication 13, American Association for the Advancement of Science, 1940, p. 295.

Ragan, Atchley and Loeb.[15] Soffer, Engel and Oppenheimer[16] saw hypertension develop in 3 patients with Addison's disease who were under treatment with desoxycorticosterone, which they thought was due to excessive dosage. Numerous investigations, recently reviewed by Page,[17] have shown that either the adrenal cortex or a hormone produced by it is essential for the maintenance of the elevation of blood pressure observed in the hypertension of experimental renal ischemia. It is significant that the adrenal cortex is an essential part of the mechanism of the maintenance of this experimental renal hypertension, even though it probably plays a secondary role. The mild hyperplasia of the adrenal cortex which we have described in cases of chronic pyelonephritis would appear consistent with the concept that there is secondary stimulation of the adrenal cortex in cases of renal hypertension.[18] While the hyperplasia of the adrenal cortex which we have shown to occur commonly with essential hypertension may also be a secondary effect, it is possible that "primary" hyperplasia and hypersecretion of the adrenal cortex might induce hypertension. On the basis of the pathologic changes and other evidence presented, investigation of this possibility might prove fruitful. The correlation of hyperplasia of the adrenal cortex with essential hypertension is almost as definite as that of hyperplasia of the thyroid gland with exophthalmic goiter.

SUMMARY

Nodular or adenomatous hyperplasia of the adrenal cortex is almost regularly found in association with essential hypertension. The cells are hyperplastic and many show a high lipoid content. Comparable changes are infrequent in adrenal glands from nonhypertensive patients. On the basis of this finding and other evidence cited, it is suggested that hypersecretion of the adrenal cortex may in some cases be a factor in the genesis of what is now classified as essential hypertension. This possibility should be investigated.

15. Ferrebee, J. W.; Ragan, C.; Atchley, D. W., and Loeb, R. F.: J. A. M. A. **113**:1725, 1939.

16. Soffer, L. J.; Engel, F. L., and Oppenheimer, B. S.: J. A. M. A. **115**:1860, 1940.

17. Page, I. H., in Blood, Heart and Circulation, Publication 13, American Association for the Advancement of Science, 1940, p. 230.

18. In light of the fact that in cases of essential hypertension arteriolar sclerosis is usually demonstrable in the kidneys at the time of death, it can easily be seen that a factor of renal hypertension would probably operate ultimately in this disease regardless of the initial vasopressor mechanism.

INCIDENCE AND LOCALIZATION OF CORONARY ARTERY OCCLUSIONS

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Publications on the incidence of occlusions in the coronary arteries have appeared for many decades, and the current literature on this topic is still extensive. The comparative frequency of such occlusions has enabled many investigators to report on relatively large series of cases from almost every conceivable angle. Throughout the literature there is a constant tendency to draw conclusions from data indiscriminately obtained from a mixture of clinical studies and postmortem examinations. Recent publications [1] have emphasized a long-standing doubt of the closeness of the correlation between the usual clinical evidence for "coronary occlusion" and the postmortem demonstration of such a lesion. The accuracy of this correlation, however, has been so frequently assumed that it colors the whole concept of coronary artery disease. The sources of this error arise first in the misinterpretation of clinical data in terms of pathologic phenomena when pathologic examination has not been made, and second in the use of inadequate methods of postmortem examination of the coronary arteries.

We have investigated this subject again, using an improved technic [2] for studying the pathologic alterations in the coronary artery system. Briefly, this method consists of (1) injecting radiopaque lead-agar masses of different colors into the coronary arteries, (2) unrolling the heart so that the entire coronary artery tree lies in one plane, (3) taking a roentgenogram of the unrolled heart and (4) carefully dissecting the coronary arteries, using the film as a guide. This technic discloses all narrowings, occlusions and anastomoses of the coronary arteries in every heart examined.

Analyses of discrepancies between the clinical and the postmortem findings on a series of human hearts thus studied have already been

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From the Pathology Laboratory, Beth Israel Hospital, and the Department of Pathology, Harvard Medical School.

1. (*a*) Saphir, O.; Priest, W. S.; Hamburger, W. W., and Katz, L. N.: Am. Heart J. **10**:567, 762, 1935. (*b*) Blumgart, H. L.; Schlesinger, M. J., and Davis, D.: ibid. **19**:1, 1940.

2. Schlesinger, M. J.: Am. Heart J. **15**:528, 1938.

published.[3] These reports emphasized the necessity of a more accurate interpretation of the clinical data. A number of the factors underlying the deficiencies in the usual postmortem examination of the coronary arteries are herein discussed.

In most studies which include postmortem observations the incidence of complete occlusions and that of marked narrowings of the coronary arteries are considered together. It is true that marked narrowing of these vessels is of extreme functional significance. As an objective quantitative observation, however, it is much less definite than is the demonstration of a zone of complete occlusion in the vessel. Almost all such complete occlusions of the coronary arteries are preceded by marked arteriosclerotic narrowing. Therefore it is believed that the conclusions reached from a study of the incidence and localization of complete occlusions could be applied to the phenomenon of marked narrowing. Hence, this study was devoted exclusively to the more definite phenomenon of complete occlusion.

AGE AND SEX DISTRIBUTION OF OCCLUSIONS OF THE CORONARY ARTERIES

In an earlier communication,[4] based on the study of 225 injected hearts, we called attention to the appallingly high incidence of hearts with coronary artery occlusions in males over 55 years of age—40 per cent. This incidence was much higher than we had anticipated from our own experience before the use of this special technic for examining the coronary artery tree. Therefore, the protocols of the 660 autopsies performed at the Beth Israel Hospital before this technic was instituted were reviewed for the incidence according to age and sex of hearts with coronary artery occlusions.

The figures for these hearts as well as for the 400 injected and dissected hearts are given in table 1. Since no coronary artery occlusions were found in the hearts of persons under the age of 20, this group was entirely omitted from consideration. In almost all the several categories of age and sex a significantly larger number of occlusions were disclosed by the injection plus dissection technic. The one outstanding exception was the group of 56 hearts from women 40 to 59 years of age. The failure to find an increased number of occlusions in this group by a technic disclosing such an increase in all other groups emphasizes anew the sharp difference in this respect between men and women in this age group.

As control material for our earlier series of uninjected hearts, there were available similar studies on uninjected hearts from two other large

3. Blumgart, H. L.; Schlesinger, M. J., and Zoll, P. M.: J. A. M. A. **116**: 91, 1941. Blumgart, Schlesinger and Davis.[1b]

4. Schlesinger, M. J.: Arch. Path. **30**:403, 1940.

Boston hospitals. At the Massachusetts General Hospital [5] 203 hearts, or 11 per cent of a total of 2,898 hearts studied, had coronary artery occlusions. Such occlusions were found in 30, or 7.5 per cent of 398 hearts studied, at the Long Island Hospital.[6] When the individual categories of age and sex for these two hospitals were compared with those in our earlier series, no significant differences were disclosed. Indeed, the figures for the total gross incidence of hearts with coronary artery occlusions in these three series of uninjected hearts do not differ greatly.

Since the findings in our series of injected hearts differed so significantly from those in this large group of uninjected hearts, it was deemed desirable to analyze these findings further. This analysis was aimed at determining why and where the older methods overlooked so

TABLE 1.—*Increased Number of Hearts with Occlusions in the Coronary Arteries Disclosed by the Injection Plus Dissection Technic*

		1929-1936 Coronary Arteries Studied by Dissection Only			1937-1940 Coronary Arteries Studied by Injection Plus Dissection		
		Total Number of	Hearts with Occlusions		Total Number of	Hearts with Occlusions	
Age	Sex	Hearts	Number	Per Cent	Hearts	Number	Per Cent
20 to 39	M	56	1	1.8	44	1	2.3
	F	62	0	0	19	1	5.3
40 to 59	M	181	17	9.4	85	20	23.5
	F	115	5	4.4	56	2	3.6
60 to 79	M	158	31	19.6	115	43	37.4
	F	75	12	16.0	65	21	32.3
80 on	M	9	1	11.1	9	4	44.4
	F	4	2	50.0	7	2	28.6
Total.....		600	69	10.5	400	94	23.5

many points of occlusion of the coronary arteries and in what particulars the distribution disclosed by the more complete data differed from the traditional distribution.

LENGTH OF OCCLUSIONS

An important source of uncertainty in arriving at a true concept of the incidence and localization of occlusions in the coronary arteries by the more commonly used methods is the difficulty of recognizing by those methods very short zones of occlusion. With the injection plus dissection technic there were recorded the exact lengths of 193 of the 198 occlusions detected in the main stems and their branches in 400 hearts. The data thus obtained are shown graphically in the chart

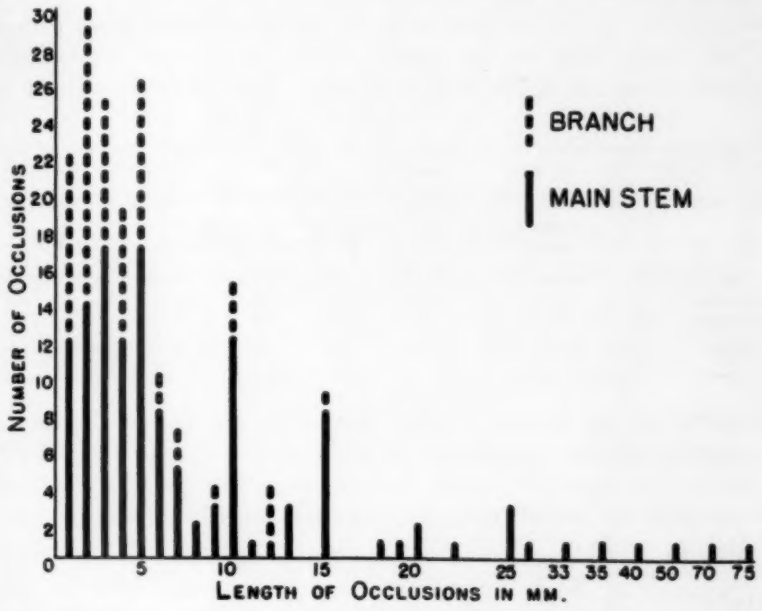
5. Gordon, W. H.; Bland, E. F., and White, P. D.: Am. Heart J. **17**:10, 1939.

6. Ackerson, I. B.; Dias, J. F., and Monroe, R. T.: New England J. Med. **217**: 622, 1937.

and are summarized in table 2. Almost two thirds of the occlusions were found to be less than 5 mm. in length, and most of these short occlusions were less than 3 mm. in length. Such short occlusions may easily be overlooked. Exploring such a spot with the point of a dissecting scissors or a probe to ascertain the completeness of the occlusion, one is likely

TABLE 2.—*Length of Occlusions in the Coronary Arteries*

	Length of Occlusions in Millimeters				
	<3	3-5	5-20	>20	Total
Number of occlusions	77	45	60	11	193
Percentage of total	40	23	31	6	100



The distribution of occlusions of various lengths in the main stems and branches of the coronary arteries of 400 hearts.

to run the point of the instrument through the occlusion, especially if it is not completely organized, as is so often true.

THE "ARTERY OF CORONARY OCCLUSION"

The left descending coronary artery has been repeatedly called the "artery of coronary occlusion and of sudden death." This artery is supposed to have an exceptional predisposition to such occlusions. Since almost all cardiac infarcts involve the left ventricle only,[7] this was

7. Mallory, G. K.; White, P. D., and Salcedo-Salgar, J.: Am. Heart J. **18**: 647, 1939. Saphir and others.[1a] Blumgart and others.[1b] Schlesinger.[4]

regarded as an expected correlation. However, the important relation of occlusions in the other coronary artery branches to infarction of the left ventricle has recently been emphasized.[8] The significance and frequency of "infarction at a distance" is now well understood. Our findings on the numbers of occlusions in the main vessel and branches of each of the three major coronary arteries again emphasize that point. These findings are presented in table 3.

Table 3 includes all the 198 healed or fresh individual occlusions in the coronary arteries uncovered in the series of 400 injected hearts. The left descending coronary artery proves to be the "artery of coronary occlusion" only in the sense that occlusions are slightly more common in the main stem and branches of that artery than in either of the other two arteries. However, occlusions are not significantly more frequent in that vessel than in the right coronary artery and its branches. Besides, there are many more occlusions distributed throughout the

TABLE 3.—*Distribution of Occlusions in the Main Stems and Branches of the Coronary Arteries*

	Left Descending Coronary Artery		Left Circumflex Coronary Artery		Right Coronary Artery		All Three	
Part of Artery	Number	Per Cent	Number	Per Cent	Number	Per Cent	Number	Per Cent
Main stem	52	39	36	27	46	34	134	100
Branches	24	37	16	25	24	38	64	100
Total	76	38	52	26	70	35	198	100

remainder of the coronary artery tree than are present in the left descending coronary artery and its branches.

MANNER OF BRANCHING AND LOCALIZATION OF OCCLUSIONS

Whitten [9] was the first to suggest that a difference in the number of occlusions in the left coronary artery as compared with the right coronary artery might be due to a difference in the manner of branching of these two vessels. He called attention to the fact that the branches of the left coronary artery leave the main stem or its larger divisions at right angles to the direction of the blood flow in the vessel. These branches then plunge directly into the thick wall of the left ventricle. The branches of the right coronary artery, however, leave the main stem in the plane of the epicardial surface and do not turn and plunge into the thin wall of the right ventricle. Because of the clarity with which the method of branching can be demonstrated in the injected

8. Bean, W. B.: Am. Heart J. **14**:684, 1937. Saphir and others.[1a] Blumgart and others.[1b] Blumgart and others.[3]

9. Whitten, M. B.: Arch. Int. Med. **42**:846, 1928.

and unrolled heart, it seemed advisable to study this question in our series of injected hearts.

One of us (M. J. S.[4]) proposed a classification of human hearts according to the basic anatomic pattern of the coronary arteries. About 50 per cent of human hearts show the right coronary artery preponderant. This group of hearts is especially suitable for studying the effect of the mode of branching on the location of the occlusions. In this group the right coronary artery first lies in the right auriculoventricular groove as far as the crux of the heart. This segment can be considered the proximal portion of the right coronary artery. Beyond the crux of the heart, the distal part of this vessel occupies the left auriculoventricular groove. The proximal part of the right coronary artery, which supplies the right ventricle, branches in a characteristic manner. In the distal part of the right coronary artery, beyond the crux, its branches supplying the thick left ventricle arise in a manner similar to the branches of the left coronary artery system.

TABLE 4.—*Incidence of Occlusions in the Main Stems of the Coronary Arteries in Two Hundred and Seven Right Coronary Artery Preponderant Hearts*

	Left Descending Coronary Arteries	Left Circumflex Coronary Arteries	Right Coronary Arteries		
			Proximal Segments	Distal Segments	Total
Total length of vessels in centimeters..	3,198	1,060	1,730	863	2,593
Number of occlusions present.....	26	19	17	7	24
Number of centimeters per occlusion..	123	55	100	123	108

Of the 400 hearts studied, 207 showed this type of right coronary artery preponderance. We measured the lengths of the arteries of each of these 207 hearts as depicted on the roentgenograms. In addition to the length of each of the three main arteries, the length of the proximal and that of the distal segment of the right coronary artery, as defined in the foregoing paragraph, were recorded separately. The total length of these four main segments of the coronary artery tree in the 207 right coronary artery preponderant hearts and the distribution of the 69 occlusions present in these segments are shown in table 4.

The incidence of occlusions per unit of length of the main stem was highest (1 occlusion per 55 cm.) in the relatively short left circumflex coronary artery. The aggregate lengths of the left descending and the right coronary arteries were respectively three and two and one-half times that of the left circumflex arteries. In neither of these two vessels was the number of occlusions proportionately increased. Less than one half as many occlusions per centimeter of length were found in these two arteries (1 occlusion per 123 cm. and 108 cm., respectively) as were found in the left circumflex artery. Thus, in this group of

selected hearts, if any artery deserved the name of the "artery of coronary occlusion," it was the short left circumflex coronary artery.

The differential study of the incidence of occlusions in the proximal and distal segments of the right coronary artery in this selected group of hearts does not support the concept that the localization of an occlusion is related to the mode of branching of that vessel. There was a slightly higher incidence of occlusions per centimeter in the proximal segment (1 occlusion per 100 cm.) of this vessel, sending branches to the right ventricle, than in the distal segment (1 occlusion per 123 cm.), sending branches to the left ventricle. The distal segment in turn presented relatively as few occlusions as the left descending coronary artery.

The branches leaving these main vessels were similarly measured. However, the number of occlusions found in the branches in this series was not large enough to warrant comparative analysis.

TABLE 5.—*Incidence of Occlusions in the Main Stems of the Coronary Arteries in Twenty-Nine Left Coronary Artery Preponderant Hearts*

	Left Descending Coronary Arteries	Left Circumflex Coronary Arteries			Right Coronary Arteries
		Proximal Segments	Distal Segments	Total	
Total length of vessels in centimeters	505	215	88	303	223
Number of occlusions present	5	2	1	3	4
Number of centimeters per occlusion	100	108	88	100	55

Twenty-nine of the 400 hearts injected constituted a small group in which the left circumflex coronary artery had distinct proximal and distal segments. These segments had connotations the opposite of those of the segments of the right coronary artery in the group discussed in foregoing paragraphs. These hearts formed only such part of the whole left coronary artery preponderant group as had a distinct segment of the left circumflex artery traversing the right auriculoventricular groove. In table 5 this small group is analyzed as was the right coronary artery preponderant group in table 4. The numbers of occlusions in the proximal and distal segments of the left circumflex coronary arteries in this group are too small for accurate comparison. Such an analysis will have to await the accumulation of a larger series. However, in this selected group of hearts the highest incidence of occlusions per centimeter of length of vessel was in the short right coronary artery (1 occlusion per 55 cm.). In these hearts the right coronary artery might have been called the "artery of coronary occlusion." Thus, the postulated relationship between the manner of branching of a coronary artery and the incidence of occlusions in the artery has not been confirmed.

DISTANCE OF OCCLUSIONS FROM MOUTH OF VESSEL

A different correlation seemed possible, however, from the observation that the shortest artery was the "artery of coronary occlusion" in both groups of selected hearts discussed. This would be explicable if the incidence of occlusions was progressively higher nearer the mouth of each vessel. This point was investigated. At the time of dissection we customarily measured and recorded the distances from the mouths of the main coronary artery stems to the points of occlusion. The site of each point of occlusion was then marked on the roentgenogram. Later the distance from the mouth of the vessel to this site of occlusion was checked by measuring it with a map measurer on the roentgenogram of the injected vessels. Such doubly checked measurements were available for 125 of the 134 occlusions found in the three main coronary

TABLE 6.—*Distribution of Old and Fresh Occlusions in the Main Stems with the Distances from the Coronary Mouths*

Artery Involved	Type of Occlusion	Occlusions Found at Given Distance from Coronary Mouth										
		0-1 Cm.	1-2 Cm.	2-3 Cm.	3-4 Cm.	4-5 Cm.	5-6 Cm.	6-7 Cm.	7-8 Cm.	8-9 Cm.m	9-10 Cm.	Total
Left descending coronary artery	Old	2	3	19	5	3	2	2	2	0	1	39
	Fresh	1	2	2	2	2	1	0	1	0	0	11
Left circumflex coronary artery	Old	3	9	4	5	2	1	1	3	0	1	29
	Fresh	1	0	2	0	0	1	0	0	0	0	4
Right coronary artery	Old	3	6	5	3	3	1	1	3	0	5	30
	Fresh	2	3	1	3	0	0	1	1	0	1	12
Total.....	Old	8	18	28	13	8	4	4	8	0	7	98
	Fresh	4	5	5	5	2	2	1	2	0	1	27

artery stems. The measurement used was the distance from the ostium of the vessel at the aorta to the proximal edge of the occlusion.

The distribution of these measurements is recorded in table 6. Eighty-six (69 per cent) of the occlusions in the main stems of the coronary arteries were found in the proximal 4 cm. of these vessels, measured from their ostiums. The incidence of occlusions in the zone nearer the mouth was high in all three main vessels, not only in the left descending coronary artery. However, in each vessel the point of highest incidence of occlusions was not directly at the mouth of the vessel but a short distance distal to the mouth.

POINT OF HIGHEST INCIDENCE OF OCCLUSIONS

The highest single concentration of occlusions was found in the zone from 2 to 3 cm. from the mouth of the left descending coronary artery, as has been repeatedly reported. This localization is supposed to be related to the origin of a large septal branch at this point. Such a large constant septal branch in human hearts is described and named both by

Spalteholz [10] and by Gross.[11] In our series of injected and dissected human hearts such a single large septal vessel arising from the left descending artery was rarely found at any level. More commonly a brush of septal branches of various sizes leaves the left descending coronary artery at various levels. Illustrations of these variations have been presented in previous publications.[12] However, in the smaller number of dog and of pig hearts we have similarly injected, we have found such a large septal vessel to be constant, as has been the experience of Piannetto [13] and of Fauteux,[14] among others. Another point against the assumed relation between the location of occlusions and the origin of the septal branch is the absence of a large constant branch leaving either the right coronary artery or the left circumflex coronary artery at the point of highest incidence of occlusions.

We can offer no satisfactory explanation of this high localization of occlusions in a narrow zone in each of the three coronary arteries. It cannot be ascribed to a greater tendency of the fresh occlusions to heal the farther from the mouths of the vessels they occur. In both the proximal 4 cm. and the more distal portions of the vessels the same percentage of the occlusions uncovered (21.5 per cent) were fresh occlusions.

COMMENT

The method used discloses in each heart studied all occlusions in all coronary arteries 1 mm. or more in diameter. All possible doubts about the presence of such occlusions are resolved before the dissection is completed. When the shadow of the injection mass in a coronary artery appears discontinuous on the roentgenogram, the presence of the suspected complete occlusion must be confirmed by the dissection of the vessel. When a true occlusion is present, the dissection will demonstrate at the suspected area the occluded lumen and the lack of tinting of the vessel wall by the injection mass. In other instances, the roentgenogram may not show clearly a complete break in the shadow of the injection mass because of calcification in the wall of the artery, but the dissection will reveal whether there is occlusion in the calcified zone. After injection of the colored mass, it is generally not difficult to prove by the subsequent dissection whether or not there exists throughout the length of a markedly narrowed zone a tinted, grossly visible tortuous channel containing an injection mass.

10. Spalteholz, W.: Die Arterien der Herzwand, Leipzig, S. Hirzel, 1924.

11. Gross, L.: The Blood Supply of the Heart, New York, Paul B. Hoeber, 1921.

12. Schlesinger (footnotes *2* and 4).

13. Piannetto, M. B.: Am. Heart J. **18**:403, 1939.

14. Fauteux, M.: Surg., Gynec. & Obst. **71**:151, 1940.

Markedly narrowed zones found by the usual methods of dissection may give rise to a doubt as to whether or not there is a point of complete occlusion. Such narrowed zones are functionally almost as inefficient as if complete obliteration of the lumen were present. Nevertheless, to classify all such doubtful zones as complete occlusions would be anatomically inaccurate. It is just as inaccurate to decide that they are merely narrowed areas because one can plough through them with the point of a dissecting scissors or a probe. Probably the uncertainty about this observation has produced much of the confusion in hitherto accepted concepts of the incidence and localization of complete occlusions in the coronary arteries. The method herein described corrects the errors in both directions, in that none of the large number of very short occluded areas is overlooked and points of marked narrowing are easily distinguished from complete occlusions. There must always be a large subjective factor in interpreting the significance of any degree of narrowing. Since zones of significant narrowing generally eventually progress to complete occlusion, only those points which were proved to be completely occluded were used in the foregoing analysis.

This analysis of the location and distribution of the coronary artery occlusions disclosed by the method of injection plus dissection reveals why so many occlusions are ordinarily overlooked. There is a general lack of appreciation of the high incidence of such occlusions in both the left circumflex and the right coronary artery, especially in their proximal 4 cm. Because the relation of these occlusions to lesions anywhere in the left ventricle has not been fully appreciated, search for occlusions in these two vessels has not been arduous enough. Also, as a routine task it is too laborious to examine the complete length of all the various branches of the coronary arteries either by making multiple cross sections or by opening the vessels with a scissors. Therefore, many of the occlusions in the smaller branches, comprising 32 per cent of all the occlusions present, are never uncovered.

In dissecting a heart, the first inch (2.5 cm.) of each of the three main coronary arteries should be closely examined for occlusions, most of which will be only a few millimeters in length. Within these 3 inches (7.5 cm.) will be found almost one half of the occlusions in the coronary arteries. It would, however, be necessary to examine just as carefully all the remainder of this large arterial tree, measuring on the average about 34 inches (about 85 cm.) in length, to demonstrate all the other 50 per cent of the occlusions.

SUMMARY

More than one half of the points of occlusion in the coronary arteries are overlooked by ordinary dissections.

Most zones of occlusion of the coronary arteries are less than 5 mm. in length and are therefore easily overlooked.

Occlusions are as numerous in the right coronary artery as in the left descending coronary artery.

There is no relation between the manner of branching of the coronary arteries and the localization of occlusions therein.

The majority of coronary artery occlusions are found within 3 cm. of the mouths of these vessels.

DISTRIBUTION OF ACID PHOSPHATASE IN THE TISSUES UNDER NORMAL AND UNDER PATHOLOGIC CONDITIONS

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Enzymes capable of hydrolyzing phosphoric esters have attracted a great deal of interest since the original demonstration of phosphatase activity in living tissues by Grosser and Husler [1] in 1912. The first observations on the presence in the human spleen and liver of a phosphatase having an optimum p_H of 4 to 5 are those of Davies [2] in 1934.

Kutscher [3] in a series of papers showed that urine contains a similar phosphatase, the origin of which could be traced to the prostate. Extremely large amounts of this acid phosphatase (from several hundred to several thousand units per gram of tissue) may be present in the adult human prostate. Acid phosphatase in many respects is considerably different from the more common alkaline variety. Its resistance to inactivating agents, its optimal p_H range and its distribution in the organs are quite different from those of alkaline phosphatase. According to all evidence available, it is a substance distinctly different and independent from alkaline phosphatase.

A method for the microtechnical demonstration of alkaline phosphatase has been described previously [4]; in the present paper an adaptation of this method to demonstrate the intracellular distribution of acid phosphatase in microscopic sections is presented.

EXPERIMENTAL METHOD

The original method, for alkaline phosphatase, is based on the formation of a calcium phosphate precipitate from calcium glycerophosphate by enzymatic action. This precipitate of calcium phosphate is practically insoluble at p_H 9, the optimum p_H of alkaline phosphatase. However, it is easily soluble at the optimum of acid

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1. Grosser, P., and Husler, J.: Biochem. Ztschr. **39**:1, 1912.
2. Davies, D. R.: Biochem. J. **28**:529, 1935.
3. Kutscher, W.: Ztschr. f. physiol. Chem. **235**:62, 1935. Kutscher, W., and Wolbergs, H.: ibid. **236**:237, 1935. Kutscher, W., and Woerner, H.: ibid. **239**:109, 1936.
4. Gomori, G.: (*a*) Proc. Soc. Exper. Biol. & Med. **42**:23, 1939; (*b*) J. Cell. & Comp. Physiol. **17**:71, 1941.

phosphatase, which is around p_H 5. Therefore it was necessary, first of all, to find a phosphate precipitate insoluble at p_H 5. Two phosphates meet this requirement, those of uranium and lead. In previous experiments on alkaline phosphatase it was found that whereas uranium at acid reaction irreversibly inactivates phosphatase, lead in moderately acid ranges is practically nontoxic to the enzyme.

That lead is not greatly toxic to acid phosphatase either was proved in the following way: Frozen sections of fresh unfixed human prostate were placed at 37 C. in a solution of sodium alphaglycerophosphate buffered with an acetate buffer at p_H 5 and containing lead nitrate in hundredth-molar concentration. In ten to fifteen minutes the solution became very turbid. The turbidity was insoluble in acetic acid but readily soluble in dilute nitric acid. This corresponds to the solubility of lead phosphate. In addition, an opaque white precipitate of the same behavior in regard to solubility settled down in the slices themselves. The gross pattern of the distribution of this precipitate in the tissue suggested a selective localization in the glands. The slices were rinsed in dilute acetic acid and transferred to a solution of hydrogen sulfide. The white precipitate immediately turned black, and microscopic examination of the slices clearly showed that only the lining of the glands was stained, the remainder of the tissue being entirely unstained.

In addition to this rather crude test, quantitative determinations were carried out on the acid phosphatase activity of prostatic fluid in the presence of lead salts. It could be shown that even comparatively large amounts of lead added to the buffered substrate-enzyme mixture will not completely suppress the hydrolysis of sodium phenyl-phosphate by the enzyme. The inhibition by lead was found to be around 25 per cent at two hundredth–molar concentration, around 35 per cent at hundredth-molar concentration and around 60 per cent at fiftieth-molar concentration.

As unfixed frozen sections are difficult to handle and to mount so as to obtain satisfactory slides, an attempt was made to fix the tissue and to embed it without destroying the enzyme.

Unfixed frozen sections were exposed to fixatives and to embedding mediums, such as solution of formaldehyde U. S. P., alcohol, dioxane (diethylene dioxide) and acetone. After various intervals of time they were removed from the fluid to be tested, rinsed in distilled water and incubated and stained as described previously. It was found that solution of formaldehyde U. S. P., alcohol and dioxane completely destroy the enzyme almost instantly. However, acetone was found to preserve both alkaline and acid phosphatase very well. This is contrary to the observation of Kutscher that acetone rapidly destroys prostatic phosphatase. The results were better when cold concentrated acetone was used. Excellent preparations were obtained even after the slices had been preserved for a week in absolute acetone at ice box temperature. Although considerable inactivation must have occurred in the case of acetone-treated tissues, as judged by the greatly increased time of incubation required, these tissues gave pictures indistinguishable from those of untreated sections, provided the time of incubation was long enough (one hour or more). Moreover, in sections dehydrated with acetone at a low temperature the enzyme became remarkably resistant to alcohol and heat. Exposure of acetone-treated sections to 95 per cent or absolute alcohol or to 65 C. for three hours did not cause any noticeable decrease in enzyme activity. Incidentally, it is remarkable how little shrinkage is caused by cold absolute acetone. Sections of tissues fixed in it compare favorably with sections of tissues fixed in the best routine fixatives.

These facts permitted the elaboration of a method for the demonstration of sites of acid phosphatase activity in paraffin sections. Experience with hundreds

of tissue blocks has shown that this method is quite reliable in yielding uniform results, provided the instructions are followed closely, especially those regarding the composition of the incubating fluid.

The method is as follows:

Fix thin slices of tissue (not more and preferably less than 2 mm. in thickness) in absolute acetone at ice box temperature for from twelve to twenty-four hours. Thicker slices will show activity at the periphery only. Change the acetone three to four times at room temperature in the course of the next twenty-four hours. Embed tissue through cedar oil followed by benzene or through benzene only in paraffin at 56 to 58 C. The tissue should not be left in paraffin for more than three hours.

Cut blocks at about 6 microns. Flatten sections in luke-warm water; in hotter water they have a tendency to go to pieces. Mount sections with egg white–glycerin and dry them.

Run sections through xylol and two changes of 95 per cent alcohol. Rinse briefly in distilled water. Transfer to the incubating solution, preheated to 37 C., and incubate for from one and one-half (human prostate) to six or more hours (other tissues). When the enzyme concentration is very low, the slides have to be incubated for about fifteen hours.

The incubating mixtures that gave the best results had the following composition:

Molar acetate buffer p_H 4.7 to 5.3	2½ to 3½ parts [5]
5 per cent lead nitrate	1 part
Distilled water	6 parts
2 per cent sodium alphaglycerophosphate	3 parts

The components are mixed in the order of mention.

This is a stock solution that will keep in the ice box for several months. If it becomes slightly turbid, it should be cleared by centrifugation, since the precipitate will pass through filter papers. Strongly turbid solutions must be discarded.

Dilute 1 part of stock solution with 2 to 3 parts of distilled water before use.

Suitable acetate buffers can be prepared in the following way: Mix 100 cc. of molar (13.6 per cent) solution of sodium acetate ($CH_3COONa.3\ H_2O$) with 100 cc. (for p_H 4.7), 50 cc. (for p_H 5.0) or 17 cc. (for p_H 5.3) of molar (6 per cent) acetic acid.

Neither betaglycerophosphate nor phenylphosphate are suitable substrates for this purpose, because their lead salts are not readily soluble in the p_H range specified.

After incubation of the sections rinse them in distilled water and then in 2 to 3 per cent acetic acid, to remove accidental precipitates (lead proteins and lead glycerophosphate). Wash thoroughly in distilled water.

The precipitate of lead phosphate that has formed in the course of the incubation is often visible as an opaque white deposit. It can be stained in one of the following two ways:

1. Transfer the section to a dilute solution of light yellow ammonium sulfide (1 cc. to a Coplin jar) for one minute. Black to dark brown (depending on the amount of enzyme present) lead sulfide will form. After thorough washing under the tap the section can be counterstained as desired.

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5. The amount of buffer required depends on the p_H of the buffer. Take 2½ parts of buffer at p_H 4.7, 3 parts of buffer at p_H 5.0 and 3½ parts of buffer at p_H 5.3.

2. Acridine red of Gruebler (but not other brands) has been found to be a very selective stain for lead phosphate. Satisfactory pictures are obtained if the slides are stained for about fifteen minutes in a mixture containing 2 parts of a 0.5 per cent solution of methyl green and 1 part of a 0.5 per cent solution of acridine red, of Gruebler. The best pictures are obtained with fresh solutions (not over two weeks old).

After staining, rinse section in distilled water, blot and differentiate shortly in 95 per cent and then absolute alcohol. Clear in xylol. Mount in balsam. Sites of acid phosphatase activity are stained a bright purplish red; the nuclei, green-blue.

To be sure, all deposits of preformed calcium phosphate–calcium carbonate that happen to be present will be stained in the same shade as acid phosphatase on account of simple chemical interaction with lead nitrate. However, they will show up even if glycerophosphate is completely omitted from the incubating mixture, whereas acid phosphatase will not.

MATERIAL

The material consisted of human and animal tissues. Some of the human tissues, normal and pathologic, were obtained at 22 autopsies (within four hours after death), while 66 various fresh specimens came from persons undergoing surgical operations. The animal tissues were those of freshly killed healthy animals (11 dogs, 7 gophers, 6 guinea pigs, 5 cats, 3 mice and 3 ground hogs).

PATTERN OF DISTRIBUTION OF ACID PHOSPHATASE IN TISSUES

Acid phosphatase is found in epithelial as well as in connective tissue elements. Sometimes secretions lying free in gland lumens give a very strong reaction. With the method used, acid phosphatase shows a diffuse distribution in the cells (either in the cytoplasm or in the nucleus or in both). It is never arranged in discrete granules.

The pattern in any given organ as observed in different individuals of the same species is fairly constant, although far less so than is that of alkaline phosphatase. Even if the localization of the enzyme in certain tissue elements is essentially identical, considerable differences in the extent and intensity of the staining are observed in different individuals of the same species. No attempts were made to correlate these variations with functional states. The same organ in different species may show either a rather similar or a different picture. The distribution of acid phosphatase, as a rule, is entirely different from that of alkaline phosphatase. To mention only the most important point, acid phosphatase is but exceptionally found in vessel walls, the most common universal site of alkaline phosphatase.

DISTRIBUTION OF ACID PHOSPHATASE IN NORMAL TISSUES

Blood and Hemopoietic System.—All blood cells of all species studied are negative for acid phosphatase both in smears and in sections. However, round cell infiltrates around strongly positive areas usually show some staining.

The spleen (fig. 1*A* and *B*) of every species studied contains heavy amounts of acid phosphatase. The enzyme is confined to the cords of Billroth, in which all cell types are stained. The darkest staining is shown by macrophages and monocyte-like elements, although lymphocytes also may show some activity. The malpighian bodies are entirely unstained except at their periphery where they merge with the cords. The trabeculae are completely negative.

Lymph glands have been found to be negative.

Digestive System, Liver and Pancreas.—In the dog, cat and rat the stomach is negative. In man it is either negative or, more often, shows some reaction in the depths of the glands. When the reaction is positive, it is practically never uniform throughout the mucosa but displays a patchy distribution, and all types of lining cells are stained. Often the parietal cells stain much paler than cells of other types. The duodenum and the small intestine usually show some staining on the surface of the villi. The pattern, although much fainter, is essentially the same as the one seen with alkaline phosphatase. At first it was thought that this faint staining might be due to the residual activity of alkaline phosphatase at *p*H 5. However, the staining is completely prevented by fluoride at two hundred-molar concentration. Alkaline phosphatase being insensitive to far higher concentrations of fluoride, whereas acid phosphatase is very sensitive, this suppression of the reaction is considered as evidence for the presence of acid phosphatase. In the large bowel there is sometimes a positive reaction in the lining of Lieberkühn's crypts, with the nuclei staining much darker than the cytoplasm.

The liver contains widely varying amounts of acid phosphatase in all species. Sometimes it is practically negative for this enzyme. In other cases only the Kupffer cells stain. Quite often, however, most of the liver cells, especially those at the periphery of the lobules, are more or less strongly positive. The bile capillaries never stand out as they do when the section is stained for alkaline phosphatase. The lining of the bile ducts is usually negative or very faintly positive.

The pancreas has been found to be negative.

Respiratory System.—No trace of acid phosphatase has been found in the lungs or bronchi of any species.

Urinary System.—The picture in the kidney is far less uniform than it is when the kidney is stained for alkaline phosphatase. Regardless of the species, the organ is quite often practically negative. Sometimes only the glomeruli are stained. In other cases the picture is very similar to that given by alkaline phosphatase; i. e., the convoluted segments of the proximal convoluted tubules are stained, although with the difference that the entire cell body gives the reaction, not just the brush border. Often the staining extends all the way down to the straight segment of the proximal

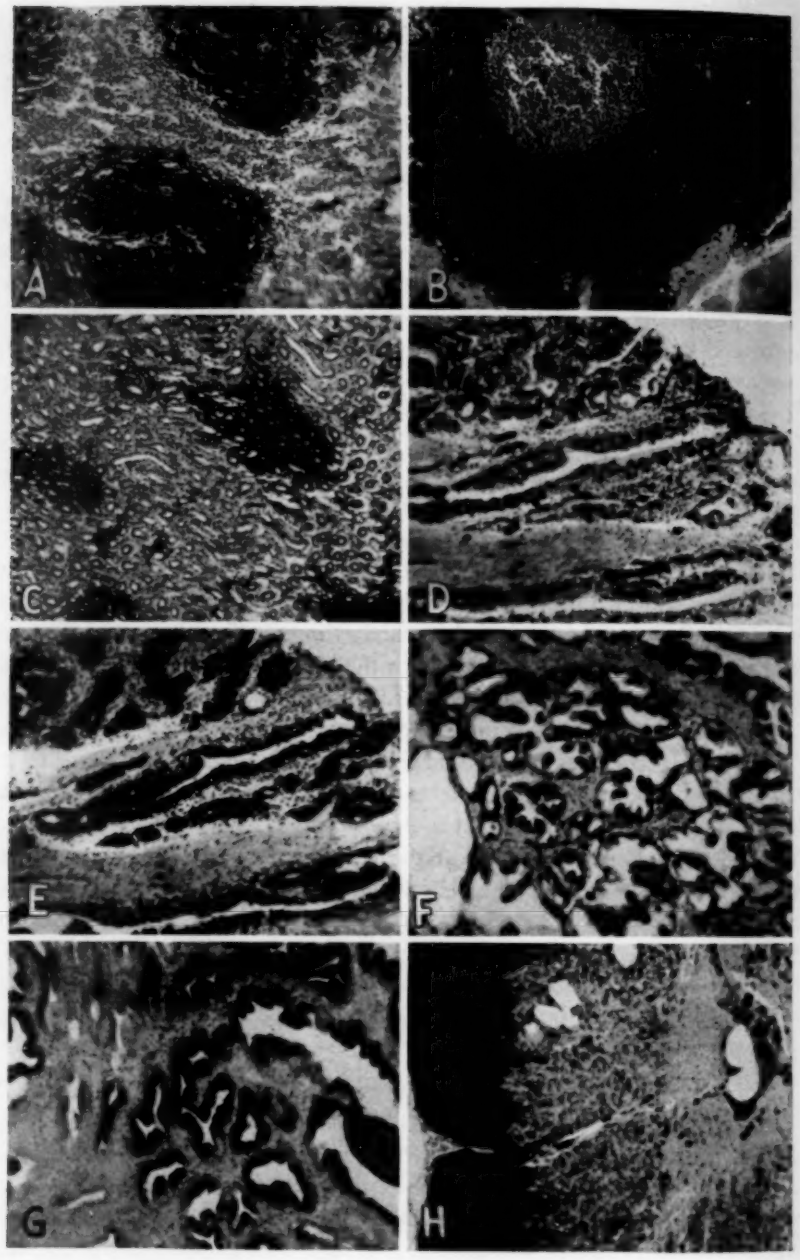

Figure 1

(See legend on opposite page)

tubules, to Henle's loops and the distal convoluted tubules, and even some of the excretory ducts may be stained. In addition to these locations, in the kidney of the cat (fig. 1*C*) groups of capillaries in the outer medulla, exactly corresponding to the structures described by Fuchs and Popper [6] as resorbent venous capillary network, are almost invariably and very selectively stained.

The epithelium of the urinary passages in the cat is positive in a spotty manner. In all other species it was found to be negative.

Male Genital System.—The tests are negative for acid phosphatase in man and in the guinea pig. In the cat the lining of the seminiferous tubules is positive. The prostate in man is very rich in acid phosphatase. Whereas alkaline phosphatase occurs chiefly in the walls of capillaries, although some may be found in the glandular epithelium, acid phosphatase is present in the glandular epithelium and in secretions within the gland lumens only (fig. 1 *D* and *E*). The amount present is so high

6. Fuchs, F., and Popper, H.: Klin. Wchnschr. **16**:1708, 1937.

General Explanation of Figures

Sections shown in figure 1 *A, D, F* and *H* were stained for alkaline phosphatase by the technic referred to in footnote 4 b. All other sections in figures 1 and 2 were stained with the sulfide variant of the technic described and counterstained with hematoxylin and eosin.

The incubation periods were: one and one half hours for figure 1 *A* and figure 2 *F* and *G*; six hours for figure 1 *B* and *G* and figure 2 *C* and *E*; fifteen hours for figure 1 *C* and *H* and figure 2 *A, B* and *D*.

The magnifications were: × 30 for figure 1 *H* and figure 2 *A*; × 70 for all others.

Explanation of Figure 1

A, spleen of dog, p_H 9.2. The malpighian bodies are positive for acid phosphatase; the pulp cords are negative.

B, spleen of dog, p_H 5. The pulp cords are strongly positive; the malpighian bodies and the trabeculae are negative.

C, corticomedullary boundary of cat's kidney, p_H 5. Groups of strongly reactive dilated capillaries may be seen.

D, human prostate, p_H 9.2. The capillaries are positive and the epithelium is negative.

E, serial section consecutive to the previous one, p_H 5, showing negative capillaries and strongly positive epithelium.

F, prostate of dog, p_H 9.2, presenting positive capillaries and negative epithelium.

G, prostate of dog, p_H 5. The capillaries are negative; the lining of most of the glands, positive.

H, adrenal of guinea pig, p_H 9.2. There is a strong reaction in the outer cortex; the medulla is negative.

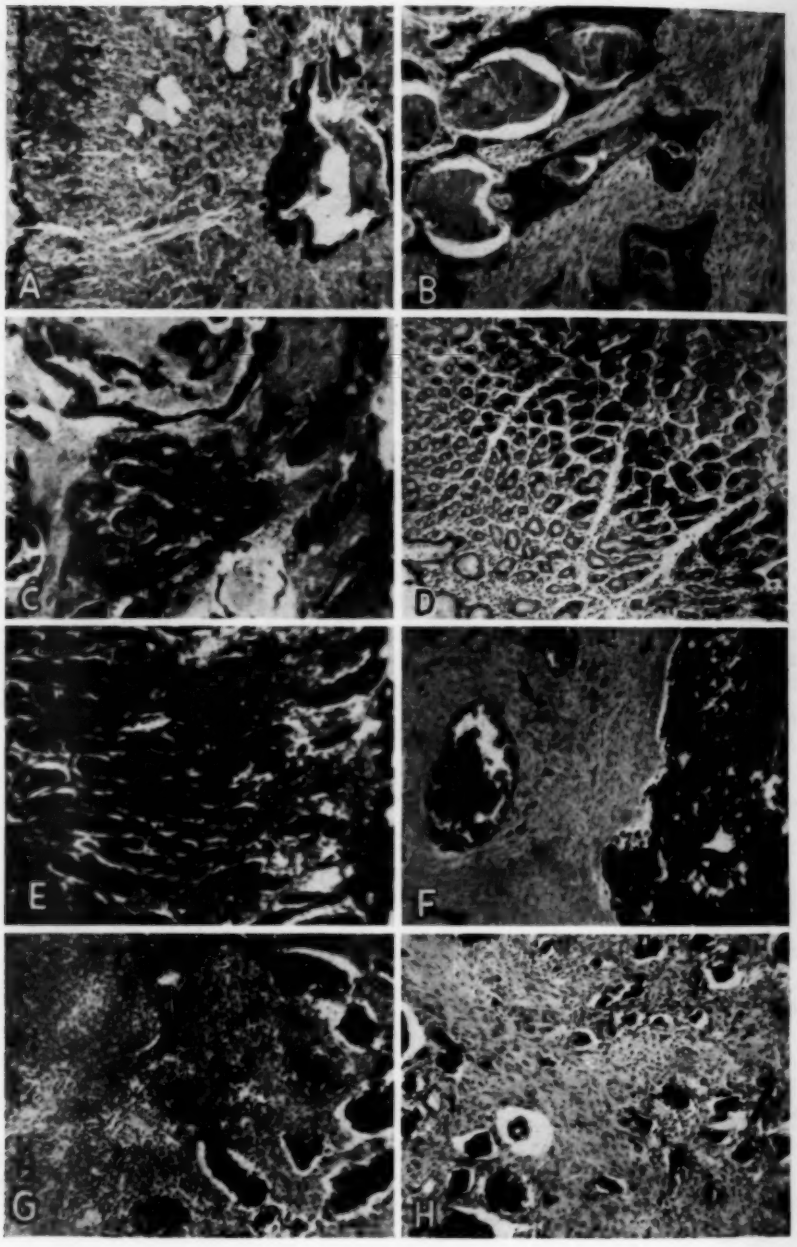

Figure 2

(See legend on opposite page)

that sometimes a reaction clearly visible with the naked eye is obtained within twenty minutes. The dog's prostate (fig. 1 *F* and *G*) contains much less enzyme, and incubation usually has to be extended to five hours or more, but the picture is essentially the same as the one seen in the human organ. Often no reaction can be obtained at all in the dog's prostate in spite of prolonged incubation. The distribution of the enzyme is not uniform throughout the organ. The lateral and ventral portions of the gland usually contain more enzyme than the dorsal portion. This applies both to man and the dog. Even within positive areas, scattered negative glands can be seen among a majority of positive ones. By staining consecutive serial sections with routine stains and for acid phosphatase, respectively, it was found that the positive do not differ morphologically from the negative glands.

Female Genital System.—This system is negative throughout, except for a slight reaction in the cells of the corpora lutea in the ovaries of the dog and the guinea pig.

Adrenal.—The pictures obtained are uniform in one respect only, and that is the practically invariably positive reaction of the medulla in all species examined. The cortex is usually, although not invariably, positive in man and in the guinea pig (fig. 1*H* and fig. 2*A*). In man the zona reticularis is more positive than the outer layers, while in the guinea pig the zona glomerulosa shows the strongest reaction. In other species the cortex is as a rule negative.

Other Tissues.—Muscles and young bones, examined without decalcification, have been found to be constantly negative. The skin and its appendages show no reaction.

EXPLANATION OF FIGURE 2

A, adrenal of guinea pig, p_H 5. A moderate reaction is seen in the cortex; a strong reaction, in the medulla.

B, bronchogenic squamous carcinoma, p_H 5. A heavy reaction is seen at the periphery of the tumor cell nests.

C, adenocarcinoma of stomach, p_H 5. Strongly positive neoplastic glands are shown.

D, mucosa of normal human stomach, p_H 5. Note moderate reaction in the depths of the glands.

E, grossly normal mucosa about 5 cm. away from a prepyloric cancer, p_H 5. An extensive heavy reaction is seen.

F, cancer of prostate, p_H 5.

G, lymph node metastasis of tumor shown in *F*, p_H 5. The tumor cell nests are positive.

H, giant cell tumor of bone, p_H 5. There are strongly positive giant cells and negative small spindle cells.

DISTRIBUTION OF ACID PHOSPHATASE UNDER PATHOLOGIC CONDITIONS

The number of pathologic specimens examined was rather small and the field of pathology covered by them rather limited; hence it is hardly possible to give more than hints about acid phosphatase pictures under pathologic conditions.

Acute Inflammations.—Areas acutely involved appear to be less rich in acid phosphatase than the normal environment. In chronically inflamed and granulation tissue, tubercles included, macrophages, degenerating epitheloid cells and giant cells quite often, though not invariably, show a strong reaction. Necrotic or caseating areas, on the other hand, are entirely free of the enzyme.

Diffuse Hematogenous Diseases of the Kidney.—No uniform changes in the distribution of acid phosphatase have been observed. It has to be remembered, however, that even the normal acid phosphatase picture of the kidney is rather variable.

Hypertrophy of the Prostate.—In some of the dilated glands both the flattened epithelium and secretions lying free in the lumens are extremely strongly stained.

Malignant Tumors.—Among the 42 tumors were 4 diagnosed as sarcoma (pancreas, 1; prostate, 1; undetermined origin, 2) which all proved to be free of the enzyme. The remaining 38 were diagnosed as carcinoma. The distribution according to the site of origin was: oral cavity, 4; bronchus, 2; breast, 4; stomach, 11; colon, 2; pancreas, 1; ovary, 3; prostate, 8; urinary bladder, 3.

Two tumors of buccal mucosa diagnosed as squamous carcinoma and their lymph node metastases showed a strong positive reaction in the degenerating central portions of the cell nests. Two other tumors that were diagnosed as squamous carcinoma showed no reaction.

One bronchial tumor classified as adenocarcinoma was moderately positive. A growth diagnosed as bronchogenic squamous carcinoma showed a strong reaction at the periphery of the cell nests (fig. 2*B*).

Carcinoma of the breast was found to be constantly negative.

With the exception of 2 growths which were negative, all tumors diagnosed as gastric carcinoma were strongly positive (fig. 2*C*) regardless of the histologic structure. The older, central portions of cell nests, which contained many necrobiotic tumor cells, were usually more active than the zones of growth. Metastases of positive tumors were also positive. Moreover, in cases of gastric carcinoma the epithelial lining of all glands throughout the entire gastric mucosa was strongly reactive. The reaction was far stronger and more extensive than in the normal stomach (fig. 2*D* and *E*).

Cancers of the large bowel showed some positive staining material in the lumens of the neoplastic glands.

Cancers of the pancreas and the ovary were negative.

In all cases except 1, carcinoma of the prostate was found to contain heavy amounts of acid phosphatase in the primary tumor as well as in its metastases, if there were any (fig. 2*E* and *G*). In a case of osseous metastases smears of bone marrow showed many strongly positive tumor cells.

The neoplastic cell nests of all cancers of the urinary bladder showed a fairly strong reaction, especially at their periphery.

In a case of giant cell tumor of the bone the giant cells were strongly positive, while the small spindle cells were negative. (fig. 2*H*).

SUMMARY

Sites of acid phosphatase activity in tissue sections can be demonstrated microtechnically by a method consisting of the following steps: Fixation in ice cold acetone. Paraffin embedding. Incubation of sections at 37 C. in a solution of lead alphaglycerophosphate buffered at p_H 5. At the sites of acid phosphatase activity a precipitate of lead phosphate will form. This precipitate is subsequently visualized by transforming it to black lead sulfide.

Acid phosphatase is an enzyme found in many normal organs. The largest amounts have been demonstrated in the human prostate and in the spleen of every species examined. As to pathologic conditions, the presence of the enzyme could be shown especially in certain malignant epithelial tumors.

In its distribution in the tissues acid phosphatase shows a typical pattern, which is entirely different from that shown by alkaline phosphatase.

OSTEITIS FIBROSA LOCALISATA

AN EXPERIMENTAL STUDY

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No agreement has yet been reached as to whether osteitis fibrosa localisata, or solitary bone cyst, is a specialized form of the more generalized osteitis fibrosa cystica or a separate pathologic entity, to be distinguished from the generalized condition by the absence of any recognizable increase of calcium or phosphorus in the blood, by the large size and definite location of the cyst and by its occurrence in the preadolescent age group. The term "cyst" is often a misnomer, for the apparent cavity in the bone as shown by the roentgenogram is frequently found to be filled by fibrous tissue, by a cluster of giant cells, by blood derivatives, by cartilage or by any combination of these elements. The center of the mass may have broken down and become liquefied, but only seldom is a true cyst, with fluid contents and a definite wall, reported. Many attempts have been made to classify the different forms. Jacoby [1] lists osteitis fibrosa localisata, brown tumor, trauma with bleeding and genuine bone cyst, the latter if only noncharacteristic tissue and purely serous fluid are present. Coley and Higinbotham [2] distinguished the true bone cyst, beginning during childhood, the giant cell variant of adolescence and the giant cell tumor, which occurs after the epiphysis is closed. Other classifications, based mostly on clinical observations, have been promulgated, but as yet none seems generally accepted.

In view of this confusion it is not surprising that many theories as to the genesis of the condition have been suggested. Konjetzny [3] stated that the cysts may follow or accompany benign tumors, subcortical or subepiphysial bleeding, degeneration of local areas of chronic osteomalacia, subchondral necrosis of the epiphysis or fracture, especially if there is delayed healing, or pseudarthrosis. Zenker [4] insisted that simple

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The theelin and parathyroid extract used in these experiments were supplied by Parke, Davis & Company.

1. Jacoby, F.: Arch. f. klin. Chir. **163**:386, 1931.
2. Coley, B. L., and Higinbotham, N. L.: Ann. Surg. **99**:432, 1934.
3. Konjetzny, G. E.: Monatschr. f. Unfallh. **46**:572, 1939.
4. Zenker, R.: Beitr. z. path. Anat. u. z. Allg. Path. **103**:451, 1939.

trauma is an essential factor but that trauma alone is insufficient and must be accompanied by some inner disturbance of the bone; he referred to the experiments of Hoffmeister [5] to show that in normal bone the simple boring of a hole is followed by complete regeneration, while in bones previously altered by large doses of roentgen radiation the same simple injury leads to cyst formation or to a blood clot with giant cells. Looser,[6] on the other hand, stated that brown tumors and cysts may appear in normal bones but may also be present in all bone diseases. He concluded that the cysts are due to blood extravasations organized, unorganized or emptied. The list of suggested causes might be extended much further.

Recent studies on the pneumatization of the humerus in the fowl [7] seem to throw new light on the causes of the cysts in human bones. The two conditions have many characteristics in common. In both, the marrow and the marrow trabeculae are dissolved and replaced by other structures, while the surrounding bone is essentially normal; in the fowl the air sac is accompanied by a type of fibrous tissue and by osteoclasts or giant cells, elements which are often found in or surrounding human cysts; and in the fowl the primary site of entrance of the sac (porus pneumaticus) is in the juxtaepiphysial region of the diaphysis, the region most commonly occupied by the solitary cyst. Moreover, although in the fowl the humerus is the only long bone to be pneumatized, in other birds the long bones so affected correspond quite accurately with those in man most commonly subject to cyst formation. In the fowl the resorption of the bone is accompanied by histologic changes exactly resembling those found in human osteitis fibrosa cystica, and the growing air sac makes its entrance through the cortex and expands in the marrow cavity always within the loose fibrous tissue characteristic of this disease.

Since the researches of Bauer, Aub and Albright [8] and of Bodansky, Blair and Jaffe,[9] osteitis fibrosa cystica has been recognized as due to a state of hyperparathyroidism. Bülbring,[10] Burrows [11] and others have confirmed this connection. But Taylor [12] found that 40 per cent of patients with parathyroid tumors may show no bone lesions, and certain clinical reports and experimental data have indicated that other hor-

5. Hoffmeister, W.: Deutsche Ztschr. f. Chir. **217**:123, 1929.
6. Looser, E.: Deutsche Ztschr. f. Chir. **189**:113, 1924.
7. Bremer, J. L.: Anat. Rec. **77**:197, 1940.
8. Bauer, W.; Aub, J. C., and Albright, F.: J. Exper. Med. **49**:145, 1929.
9. Bodansky, A.; Blair, J. E., and Jaffe, H. L.: J. Biol. Chem. **88**:629, 1930.
10. Bülbring, E.: Arch. f. exper. Path. u. Pharmakol. **162**:209, 1931.
11. Burrows, R. B.: Am. J. Anat. **62**:237, 1938.
12. Taylor, H.: Brit. J. Surg. **22**:561, 1935.

monal disturbances may cause similar changes in bone. Parhon and Tomorug [13] reported a case in which the disease was relieved after removal of an ovarian cyst. Albright and associates [14] described a syndrome characterized by osteitis fibrosa and precocious puberty in the female. Cyclic changes in the bones of female birds at egg-laying periods (Keyes and Potter [15]) and of female rodents following estrus (Gardner [16]) helped to focus attention on estrogen and progesterone as causative agents. It seems probable that the pneumatization of avian bones is due to the large amount of estrogen contained in the yolk, which is retracted into the intestine just before hatching. Though the earlier administration of estrogens to mammals often produced "marble bone" disease instead of osteitis fibrosa, bone deposition instead of bone absorption, Selye [17] has pointed out that these two conditions may be different phases of the same process; the antepartum changes in rodents consist of the softening of the pelvic bones and the simultaneous hardening of other parts of the skeleton. Recently Silberberg and Silberberg,[18] reporting experiments on young guinea pigs, described widespread absorptive processes in the cortex and trabeculae of the bones after administration of an estrogen; in other words, estrogens, as well as parathyroid extracts, can cause osteitis fibrosa.

Whether the estrogens act directly on the bone or only secondarily through the mediation of the parathyroid glands remains in doubt. The available data on this question refer only to the rise of the blood calcium level, a common concomitant of the bone disease. Altmann and Hutt [19] found that estrogens affect the parathyroid glands and that the activation of these glands results in the higher level of serum calcium characteristic of laying fowls (the free calcium being utilized in the formation of the eggshell), and Riddle and co-workers [20] showed that estrone (theelin) is wholly incapable of raising the serum calcium in parathyroidectomized rats. On the other hand, Levin and Smith [21] claimed that no significant changes of calcium level followed administration of estrone in normal rats, rabbits and monkeys except a transitory increase after huge doses. The normal range of calcium values is so variable and the normal size

13. Parhon, C. I., and Tomorug, E.: Wien. Arch. f. inn. Med. **27**:237, 1935.

14. Albright, F.; Butler, A. M.; Hampton, A. O., and Smith, P.: New England J. Med. **216**:727, 1937.

15. Keyes, P., and Potter, T. S.: Anat. Rec. **60**:377, 1934.

16. Gardner, W. U.: Am. J. Anat. **59**:459, 1936.

17. Selye, H.: Endocrinology **16**:547, 1932.

18. Silberberg, M., and Silberberg, R.: Arch. Path. **28**:340, 1939.

19. Altmann, M., and Hutt, F. B.: Endocrinology **23**:793, 1938.

20. Riddle, O., and others: Endocrine Studies, in Annual Report of the Director of the Department of Genetics, Carnegie Institution of Washington, 1938, p. 52.

21. Levin, L., and Smith, P. E.: Endocrinology **22**:315, 1938.

of the parathyroid glands in various animals and at various ages is so little standardized that slight changes may well escape attention.

In order to study more in detail the cellular changes which take place after administration of estrogen, the present experiments, closely paralleling those of Silberberg and Silberberg,[18] were undertaken. Young white rats were treated with theelin in varying amounts and over varying periods. Individual rats showed great differences in the type and extent of the response. The results noted were practically the same as those described after the administration of parathyroid extract. As expressed by Burrows:[11]

> The first histological evidence of the beginning of osteitis fibrosa is the changing of osteoblasts from their normal shape to a spindle shape. This consists of an elongation of the cells and a uniting of neighboring cells by connecting strands, the whole forming a loose syncytium not unlike mesenchyme.

Silberberg and Silberberg[18] also recognized the mesenchymal character of this tissue after the administration of theelin. The process is one of dedifferentiation by which the osteoblasts are changed back to the mesenchymal cells from which they originally developed. That the tissue so formed is truly mesenchymal or at most only slightly modified by the presence of a few collagen fibrils is proved by the use of the Mallory or azan stains. The changes are first noticeable on the peripheral bone spicules just beneath the inner cellular layer of the periosteum in the subepiphysial region, and are accompanied by absorption of the newly formed bone and local increase of osteoclasts; and this process spreads inward along the trabeculae. The initially mesenchymal nature of the invading tissue derived from the former osteoblasts should be especially noted.

As will be shown elsewhere, the same process takes place normally in growing long bones and leads to the molding or shaping of the slender shafts beneath the wider, broader articular ends. Normally the destruction of bone is strictly limited, taking place in an orderly manner along wide curves that sweep from the edge of the broad epiphysis to the surface of the narrower shaft, cutting diagonally across the tips of the newly formed bone trabeculae, which are oriented in a vertical direction following that of the vertical rows of cartilage cells in the epiphysial line. The extent and regularity of the undercutting are apparently governed by a true balance between estrogen and androgen, which are normally present in both male and female individuals. These two principles are known to be mutually antagonistic in their action on the genital system and elsewhere, and Gardner and Pfeiffer[23] have shown that this antagonism is

22. Footnote deleted by author.

23. Gardner, W. U., and Pfeiffer, C.: Proc. Soc. Exper. Biol. & Med. **37**: 678, 1938; **38**:599, 1938.

expressed also in the action of the two hormones on bone, for they were able to inhibit the action of estrogen on mice by injecting weekly 1.25 or 2.5 mg. of testosterone (oreton). In theelin-treated rats the balance is disturbed, and the destruction becomes irregular and excessive. The process spreads unevenly from the inner cellular layer of the periosteum, and its influence is felt beyond the region of the normal molding activity. In the definite cortex of the shaft it may cause the enlargement of an individual haversian canal by erosion of its walls; in the cancellated bone it may extend along a group of trabeculae, forming a broad indentation (figs. 1, 2 and 3) or a narrow plug of mesenchyma extending far into the normal bone. Wildermuth,[24] in discussing pneumatization in the bird, described this condition as reminiscent of the penetration of a neoplasm. The same picture is often seen after administration of parathyroid extract (fig. 4) and is recognizable as the initial stage of osteitis fibrosa cystica. The disease, then, is not due to an abnormal process but merely to an abnormal excess of a process which is itself normal in the molding of young bones. The occurrence of the disease in adult life can be explained as due to the reawakening of the cellular layer of the periosteum, which always retains its power of growth and its mesenchymal potentialities, as is shown by its ability to form the dense fibrous tissue of callus and to supply osteoblasts for the formation of new bone after fractures.

Later stages of the disease osteitis fibrosa are marked by spread of the bone destruction and invasion of the marrow spaces. The invading mesenchymal tissue assumes a fibrous character. This condition, however, depends on continued activation and in certain instances may not be attained. The progress may be arrested in its initial stage in either of two ways: experimentally by the use of androgens, and less drastically by terminating the estrogen treatment and allowing time for the resumption of the normal hormonal balance. That the former method is possible has been shown by Gardner and Pfeiffer[23]; that time alone may arrest the process is indicated by the present observations.

Figures 5 and 6 represent comparable sections of the jaw bones of two young fowls, twenty-one and thirty-eight days, respectively, after hatching. An air sac, in this instance a long tubular derivative of the tympanic cavity, is attempting to enter the bone by the usual method of following a mesenchymal pathway of local bone destruction, similar to those seen in the rat after administration of theelin, but due in this case to the normal absorption of yolk as before explained. At twenty-one days the process is active; at thirty-eight days activity has nearly ceased. The bone around the air sac in the older chick has resumed its normal appearance, and periosteal bone growth has almost buried the

24. Wildermuth, H. A.: Ztschr. f. Med. u. Naturwissensch. **11**:537, 1877.

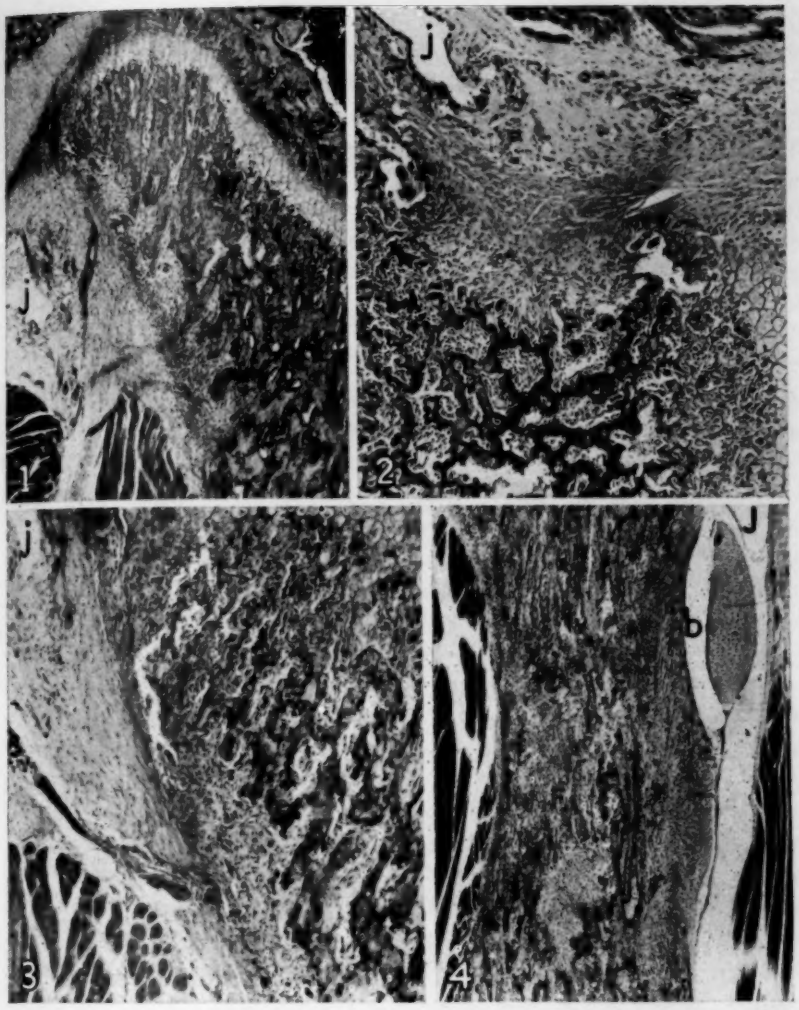

Fig. 1.—Sagittal section of a tibia of a rat 54 days old which received 50,000 international units of theelin during three weeks. Massive peripheral bone destruction. Joint cavity (*j*) enlarged by repeated trauma and injection of saturated salt solution. Vessel passing through fibrous periosteum near tip of joint cavity. × 29.5.

Fig. 2.—Transverse section near epiphysial line of a humerus of a rat 56 days old which received 10,500 international units of theelin in twenty-four days. Broad invasion of bone by mesenchymal tissue. Joint cavity (*j*) normal. × 29.5.

Fig. 3.—Frontal section of a humerus of a rat 50 days old which received 4,500 international units of theelin in twenty-one days. Broad mass of mesenchyma; destruction of bone trabeculae; osteoclasts; vessel with loose adventitial tissue passing through fibrous periosteum; normal joint cavity (*j*). × 66.5.

Fig. 4.—Frontal section of a tibia of a rat 50 days old which received 250 international units of parathyroid extract in two days. Joint cavity and bursa (*b*) distended by salt solution. × 29.5.

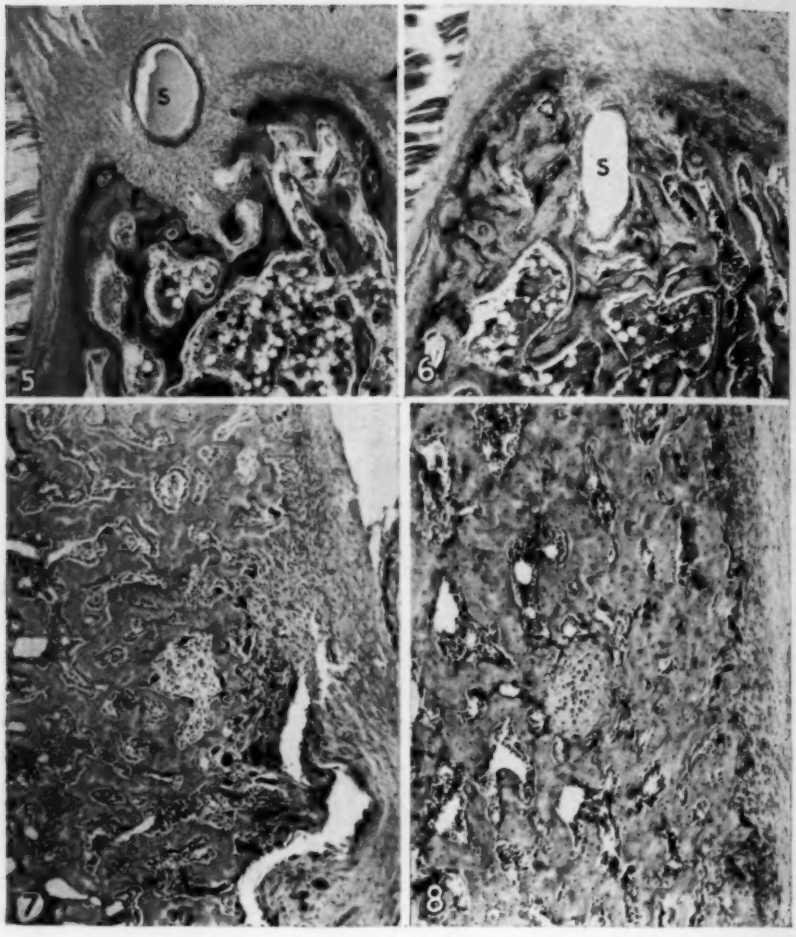

Fig. 5.—Mandible of a chick 21 days old. Air sac (*s*) entering bone preceded by mesenchyma. × 55.5.

Fig. 6.—Mandible of a chick 38 days old. Bone destruction has halted, and air sac (*s*) is becoming enclosed by normal bone growth. × 55.5.

Fig. 7.—Transverse section near the proximal epiphysis of a humerus of a rat 59 days old which received 23,600 international units of theelin in eleven days and was then sixteen days without treatment. Isolated mass of mesenchyma in cancellous bone buried by new cortex. × 66.5.

Fig. 8.—Transverse section near the proximal epiphysis of a humerus of a rat 59 days old which received 23,600 international units of theelin in eleven days and was then sixteen days without treatment. Isolated mass of cartilage buried beneath new cortex. × 66.5.

invading structure. The cessation of bone destruction may well be due to the fact that the excess of estrogen has been depleted in the older chick; some corroboration for this suggestion is afforded by the fact that the change from the early female plumage of male chicks to the definitive male plumage takes place between these two ages. The normal balance for bone growth has been restored. The air sac which entered the bone during estrogen excess cannot complete the pneumatization but remains inactive, surrounded by normal bone. A similar cessation of destructive activity and return to normal conditions were reported by Rutishauser, Broccard and Bianchi [25] in rats receiving only a single dose of parathyroid extract, but in this case the change was much more rapid because the causative agent was more powerful. The result of a single dose could be detected in four hours; after six hours the osteoclasis diminished, and after twenty-eight hours the bones showed normal histologic structure. Bülbring [10] also noted that in rats the effects of very small doses of parathyroid extract remained limited strictly to the subepiphysial region. The conditions in the older chick remind one directly of the solitary cysts found in man, and the thought was inevitable that the two might be due to the same sequence of causes and that the question might yield to experimentation.

In an attempt to reproduce in the bones of the rat conditions similar to those in the older normal chick, young white rats just weaned were treated with varying amounts of theelin for ten to twenty days and then allowed to live without injections for a further variable period, up to sixteen days. At the weaning age the rat, if one may judge by its ability for self support, can be considered comparable to the newly hatched chick. The administration of estrogen to the rat was intended to represent the absorption of yolk in the chick and the further rest period without treatment was added to allow the effect to wear off. The timing was purely a matter of conjecture. In most of the animals the initial stages of osteitis fibrosa were still present to a certain degree, indicating continuing estrogenic activity. In the bones of a few rats significant changes were found, which, while not proving that the rare human solitary cyst may be due to a similar train of events, at least suggest a new possibility among the etiologic factors.

In the subepiphysial region of one humerus, a small area of mesenchymal tissue was discovered, separated from the inner layer of the periosteum by a sheet of appositional cortex (fig. 7). As traced in serial sections, the mesenchymal mass was isolated except for a small pedicle bearing minute blood vessels. Several osteoclasts or giant cells were still present. In the same region of the humerus of another rat (fig. 8) a small mass of cartilage was present, lying deeper within the

25. Rutishauser, E.; Broccard, R., and Bianchi, M.: Presse méd. **43**:789, 1935.

shaft and entirely isolated, as proved by the study of serial sections. The cartilage might be explained as a residue of the epiphysal cartilage, which for some reason had remained in a normal state while the surrounding portions were undergoing the usual degeneration, calcification and absorption coincident with bone growth. The rounded shape of the mass and the normal condition of its cells, with no indication of the arrangement in columns or enlargement usually found in the epiphysial line, seem to me to argue against this idea. In connection with the condition found in the other bone, another theory is more probable, namely, that with the cessation of estrogenic influence a finger-like portion of the mesenchyma has been cut off by the growth of new bone and has then continued its differentiation, in this instance toward cartilage formation. McMaster [25a] has described similar cartilaginous inclusions in cases of healed rickets. The relation of cartilage and the "fibrosis" caused by the action of estrogens has been noted by Silberberg and Silberberg.[18] In their words: "The connective tissue may penetrate rather deeply into the bone and there form small plugs surrounded by bone. In these areas . . . cartilage is frequently found." In at least two of the bones of another rat of my series young cartilage was present within enlarged haversian canals. The presence of the surrounding bone may influence the differentiation of the mesenchyma in this direction. That cartilage is often found in human solitary cysts is significant. Since all the tissues described in human cysts are mesenchymal derivatives, the trapping, so to speak, of a mesenchymal plug within the bone may well give rise to the solid form of cyst.

Any small mass of tissue in this highly vascularized subepiphysial region would tend to increase in size and by pressure to erode the surrounding soft new bone. It might remain dormant while the bone grew in length. This would explain the occurrence of a cyst in the shaft of the bone as occasionally described in a patient of adolescent age. Konjetzny [3] described a cyst of the tibia, discovered after trauma in a patient of 25 years, which was located 10 cm. below the joint. The absence of any increase in serum calcium, often considered a criterion distinguishing solitary cyst from general osteitis fibrosa, would be expected since the formation of the cyst depends on the cessation of bone destruction.

The true cyst might arise by the degeneration and central liquefaction of such an isolated mass or by the erosion of vessels and consequent hemorrhage. The condition in the jaw of the older chick cited, together with the clinical reports of a few cases of the localized disease, offers another explanation. Jacoby [1] described a cyst in the upper part of the

25a. McMaster, P. E.: J. Bone & Joint Surg. **17**:373, 1935.

humerus of a child of 3 years connected with a swollen bursa beneath the shoulder muscles. Roentgen films showed a gap in the thin cortex. Ducrocq [26] reported a case in which the cyst was empty, having discharged its contents into the surrounding soft parts; this seems to indicate some hole through the cortex. Christeller [27] showed a hole leading to the central cavity in a case of the disease in a macaque. Jacoby's case clearly indicates to my mind that the bursa, under the influence of internal pressure, presumably due to some injury causing chronic bursitis, had expanded into the bone along a mesenchymal pathway provided by a concomitant temporary excess of estrogens, just as does the air sac in the humerus of the normal chick. Prevented from spreading through the whole bone by the termination of the "fibrosis" and the return of the bone to normal growth, the process of the bursa might remain as a diverticulum as in Jacoby's case, or, if the connecting isthmus were severed by the growth of new cortex, as a true cyst. Its lining and fluid contents would resemble those of bursae or joints, an observation fairly common in reported cases.

Attempts to reproduce experimentally in the rat the conditions described in Jacoby's case have failed, but the results nevertheless are interesting. Bone fibrosis was induced by the administration of theelin for two or three weeks or of parathyroid extract for two or three days (following Burrows' technic), and at the same time the knee joints were repeatedly submitted to trauma or the joint cavities filled with saturated salt solution, with the intention of increasing the fluid pressure within the joints and thus expanding their walls. The joint cavities were treated instead of bursae because they were more definite structures and therefore more readily attacked; in 1 case (fig. 4) a neighboring bursa was also swollen. Neither of the two methods used seemed adequate to maintain a continuous intracapsular pressure, for, although the cavities were often found to have extended beyond their normal limits, the walls were usually folded, showing no signs of present distention. In a few cases the cavities reached to a level opposite the invasion of the fibrosis into the bone (figs. 1, 2 and 3) but remained outside of the dense layer of the periosteum. In the bird a pathway through this outer layer is provided by the emergence of blood vessels, along whose loose adventitial coat the air sac finds its way to the mesenchyma within. A similar pathway is indicated in figures 1 and 3, and its presence in the critical position may be a requisite condition for entrance into the bone. Once within the mesenchymal tissue of the inner periosteal layer and its extension within the bone, the cavity could expand readily.

26. Ducrocq, J. L. G.: Contribution à l'étude de l'ostéite fibrokystique localisée des os longs, Thesis, no. 19, Lille, 1925-1926.

27. Christeller, E.: Ergebn. d. allg. Path. u. path. Anat. **20**:1, 1923.

Though in no case was joint cavity or bursa found within the bone, these experiments have shown that the normal limits of such cavities can be expanded by internal pressure, that "fibrosis" in its initial stages is readily established in bones by excess either of estrogen or of parathyroid hormone, and that loose perivascular tissue is often present in a position to afford a pathway for the swollen joint cavity or bursa through the dense outer layer of the periosteum to the mesenchymal tissue within. The proper conjunction of these three elements might readily result in one of the recognized types of solitary bone cyst.

SUMMARY

Previous study of the pneumatization of the bones of birds and of the influence of estrogens on the shape of mammalian long bones led to reexamination of the human diseases osteitis fibrosa cystica and osteitis fibrosa localisata. From a series of experiments on rats the following possibilities may be inferred: The general disease may be caused by a long-continued excess of estrogen, probably acting through the parathyroid glands. The local cyst may result from an excess of estrogen that is soon reduced sufficiently to permit renewed normal growth. In this case a small amount of the mesenchymal tissue, which is the initial stage of the "fibrosis," may be cut off within the bone and there undergo any of the changes met with in normal histogenesis, especially those leading to fibrous tissue and cartilage. This condition has been reproduced experimentally. Such an enclosed mass might be expected to enlarge, erode vessels, degenerate and liquefy; giant cells might cluster around it as around a foreign body. It might remain dormant for years. As an unusual variant of this more solid type, the true bone cyst could result from the rare combination of a temporary excess of estrogenic or parathyroid activity with chronic internal pressure in a neighboring joint or bursa. The condition would then resemble almost exactly that encountered in the pneumatization of avian bones. Successful entry of a diverticulum of a joint or a bursa into the bone might depend on the presence of emergent blood vessels whose loose mesenchymal adventitia could afford a pathway through the dense outer layer of the periosteum. Attempts to reproduce experimentally the proper combination of these circumstances have not been successful, but the possibility of such a combination should be considered in any attempt to explain the origin of the human solitary bone cyst.

EFFECT OF CELLOPHANE PERINEPHRITIS ON THE GRANULAR CELLS OF THE JUXTA-GLOMERULAR APPARATUS

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The increased number of certain fuchsinophilic granular cells in the juxtaglomerular apparatus in rabbits made hypertensive by partial ligation of the renal artery has been interpreted as indicating that these cells may secrete a renal pressor principle.[1] If these granular cells are the source of some constituent of the renin-vasopressor-inhibitor system, their behavior should be similar in all instances of experimental renal hypertension, however produced. And their behavior should be in accord with the recent work indicating an increase of renin and a decrease of inhibitor in hypertensive animals.[2]

Since there has been no report on the granular cells in hypertension produced by cellophane perinephritis (method of Page[3]), a study has been made of these cells in this type of hypertension and the time relations of their behavior compared with those of the reported assays on renin and inhibitor in hypertensive animals.

MATERIAL AND METHODS

Thirty-two rabbits were divided into three groups. Both kidneys of the 14 rabbits in group 1 were wrapped in cellophane. In group 2, consisting of 12 rabbits, one kidney was wrapped in cellophane and the other either was left undisturbed or was merely freed from the perirenal fat for examination. The 6 rabbits in group 3 had one kidney wrapped in cellophane and the other removed. The kidneys of 22 control animals from previous work and the kidneys of animals in group 2 served as controls for these experiments.

Blood pressures were determined by the ear capsule method of Grant and Rothschild[4] on every third day, except in experiments of short duration, in which daily readings were taken. The rabbits were killed at various intervals of time after application of the cellophane. The kidneys were fixed in Zenker's base fluid plus 10 per cent formaldehyde U. S. P., sectioned at 4 microns and stained according

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1. Goormaghtigh, N., and Grimson, R. S.: Proc. Soc. Exper. Biol. & Med. **42**:227, 1939. Dunihue, F. W., and Candon, B. H.: Arch. Path. **29**:777, 1940.

2. (*a*) Page, I. H.: Am. J. Physiol. **130**:22, 1940. (*b*) Page, I. H., and Helmer, O. M.: J. Exper. Med. **71**:495, 1940.

3. Page, I. H.: Science **89**:273, 1939.

4. Grant, R. T., and Rothschild, P.: J. Physiol. **81**:265, 1934.

to Goldner's [5] modification of Masson's trichrome stain or with iron hematoxylin. In order to facilitate observation of the granular cells, the sections were treated with 0.85 per cent hydrochloric acid in 70 per cent alcohol for twelve hours prior to staining with the trichrome mixture.

Summary of Experiments

	Group 1				Group 2				Group 3			
		Blood Pressure *		Percentage of		Blood Pressure		Percentage of		Blood Pressure		Percentage of
Time, Days	Animal	1	2	Granular Cells †	Animal	1	2	Granular Cells ‡	Animal	1	2	Granular Cells
2	75	68	68	R 12 f¶ L 11 f	76	70	70	NR 3 L 13 f	..	..	..	.....
3	72	64	67	R 25 f L 30 f	77	68	68	NR 6 L 30 f	..	..	..	.....
4	73	80	85	R 30 f L 32 f	..	..	..	.....	78	70	70	NR 2 L 26 f
5	..	..	..	.....	74	70	70	NR 32 f# R 38 f	79	74	74	NR 4 L 36 f
7	..	..	..	.....	..	..	..	.....	56a	60	60	NL 3 R 43 f
									57a	62	62	NL 5 R 32 f
14	66	80	116	R 40 m L 30 f	56	66	80	NR 4 L 45 f	..	..	..	.....
					57	70	70	NR 7 L 48 f				
21	68	78	88	R 29 f L 34 f	59	80	118	Died; no count	..	..	..	.....
28	60	80	120	R 39 h L 54 h	51	68	82	NR 2 L 74 h	..	..	..	.....
	67	84	108	R 16 h L 38 f								
35	62	70	100	R 37 h L 36 h	53	80	98	NR 8 L 50 h	..	..	..	.....
	65	84	96	R 38 f L 48 h								
49	71	78	120	R 37 f L 13 f	..	..	..	.....	..	..	..	.....
64	..	..	..	.....	61	70	90	NR 2 L 36 h	..	..	..	.....
77	58	68	80	R 3 L 50 h	..	..	..	.....	..	..	..	.....
98	..	..	..	.....	49	70	80	NR 1 L 38 f	50	78	130	NR 2 L 50 m
105	69	76	100	R 50 m L 38 m	..	..	..	.....	..	..	..	.....
	70	74	160	R # L 20 f								
168	48	72	114	R 39 f L 25 f	63	78	82	NR 10 L 59 f	..	..	..	.....
210	..	..	..	.....	47	76	86	NR 12 L 48 f	52	78	130	NR 5 L 23 f

* Column 1 gives the pressure before wrapping a kidney (or in group 1, both kidneys) in cellophane; column 2, after wrapping a kidney in cellophane.
† The percentage of granular cells in control animals is as follows: average 5; range 0-12.
‡ R = right kidney wrapped in cellophane; L = left kidney wrapped in cellophane.
§ NR = normal right kidney; NL = normal left kidney.
¶ f = few cells; m = moderate number of cells; h = great many cells.
# There was infection of one kidney.

As a basis for comparison of the numbers of granular cells in the kidneys of different animals, the following procedure was adopted: Two hundred glomeruli with identifiable juxtaglomerular apparatus were counted along the outer cortex

5. Goldner, J.: Am. J. Path. **14**:237, 1938.

of sections taken from two levels of each kidney. Whenever an apparatus contained granular cells, this fact was recorded and the number of such cells approximated by classifying them as few, moderate or many. The percentage of apparatus containing granular cells qualified by one of the foregoing adjectives served as an index of the granular cells in each kidney. Although this is not a quantitative measure, it is believed to be a reliable indication of the trend.

Attempts were made to ascertain the solubility of the fuchsinophilic granules by treating thin fresh slices of kidney for one-half to one hour with the following solutions prior to fixation: 0.85 per cent, 2 per cent and 10 per cent sodium chloride, 0.1 per cent acetic acid, 0.1 per cent sodium hydroxide, 10 per cent sucrose, 5 per cent dextrose, 1 per cent urea and 2.5 per cent glycerin. The tissues were subsequently fixed and stained in the usual manner.

In order to check the mitotic activity, if any, of the granular cells, colchicine was injected into some of the animals eight hours before killing.[6]

RESULTS

The results of this study are shown in the table. It is to be noted that the three experimental groups showed comparable increases in granular cells and that these increases were evident prior to any marked elevation of blood pressure. The elevation of pressure was not great in any group but in general was more pronounced in the groups without a normal kidney (groups 1 and 3). The cells appear to attain their maximum development in number, distribution and density of granulation within four to five weeks after the application of the cellophane, at which time the blood pressure is definitely elevated (fig. 1). Of course, this interval of time is not invariable (see animals 66 and 67); it probably varies somewhat with the degree of approximation of the cellophane to the kidney. A direct parallel between the number of granular cells and the height of blood pressure is not apparent. After fourteen weeks the number of glomeruli containing granular cells in the juxtaglomerular apparatus is still high, but the number of granular cells in each apparatus is decreased. This trend is more marked at twenty-four weeks and is quite pronounced at thirty weeks. In this later period the granules are fine and very sparse and in animal 52 the number of granular cells did not greatly exceed the normal. The juxtaglomerular apparatus of animals at these later stages contained mostly agranular cells, which appeared to be reduced in size (fig. 2).

Sodium hydroxide was the only reagent among the various ones used which appeared to alter the number of granular cells present. It is believed that this action was a blocking of the stain rather than a dissolution of the granules. This seems especially likely in view of the fact that acid treatment prior to staining intensifies the staining of the granules.

6. Colchicine was supplied by Dr. D. F. Robertson, of Merck & Company, Inc.

Mitosis of the granular cells has never been observed, although these cells are not infrequently binucleate. Division of the agranular cells of the juxtaglomerular apparatus has been seen in only 2 animals. It appears, therefore, that there is no active mitotic division of either the granular or the agranular cells of the juxtaglomerular apparatus

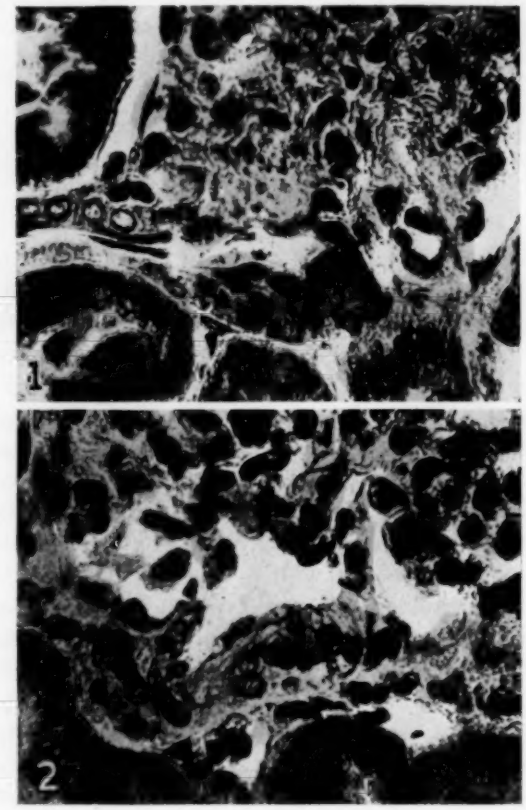

Fig. 1.—Juxtaglomerular apparatus of rabbit 65. Note the compact group of densely granular cells.

Fig. 2.—Juxtaglomerular apparatus of rabbit 52. Note the absence of densely granular cells.

COMMENT

The data presented show that the granular cells of the juxtaglomerular apparatus in response to cellophane perinephritis increase slightly in number as early as the third day after application of cellophane to the kidney, attain their maximum development at about the time that elevation of blood pressure becomes evident and thereafter gradually decrease in number. The logical question arises as to how

this behavior parallels the results of biologic assays of the various constituents of the renin-vasopressor-inhibitor mechanism. Of these constituents renin and inhibitor, or hypertensinase, appear to be formed chiefly, if not solely, in the kidney.[7] The inhibitor is reported to be decreased in experimental hypertension[7]; hence, if the granular cells are the source of inhibitor, their early increase in cellophane perinephritis indicates a blockage of secretion. The histologic evidence does not suggest this type of behavior; rather the granular cells appear to be actively secretory. Furthermore, if these cells secrete inhibitor, they should be increased either in activity or in number in the normal kidney of animals with one ischemic kidney; for the protective action of a normal kidney under such conditions is well known and is assumed to indicate inhibitor activity.[8] However, the unwrapped kidneys of the animals in group 2 in these experiments did not show such activity. These facts seem to argue against the possibility that the granular cells secrete an inhibitor. By elimination, this leaves renin as the probable secretion of the granular cells, if they indeed secrete a constituent of the renin-vasopressor-inhibitor mechanism. Biologic assays of renin in hypertensive animals do not seem to contradict this possibility. Page[2a] reported that renin assays for 3 dogs with malignant hypertension showed this substance greatly reduced as compared with assays in earlier stages of hypertension. And Kohlstaedt, Page and Helmer[9] have shown that renin is increased in the blood of animals subjected to experimental procedures which subsequently produce hypertension, prior to any marked elevation of blood pressure. The protocol for animal 418 of Kohlstaedt, Page and Helmer[9] indicates that renin formation in this animal rose to a maximum three weeks after ligation of the renal artery and then showed some tendency to decline. It seems, therefore, that the behavior of the granular cells in cellophane perinephritis is consistent with the suggestion that they secrete renin.

If the granular cells do secrete renin, the data presented indicate that after a certain period of action by renin or its reaction products the production of renin decreases but the elevation of blood pressure persists. This suggests that the arterial musculature becomes hypersensitive to the renin complex or that other mechanisms operate in maintaining hypertension or that the arterial musculature becomes sensitized to nervous or chemical stimuli or both. An increased sensitivity of the arterial system to nervous and chemical stimuli seems most likely,

7. (*a*) Muñoz, J. M.; Braun-Menendez, E.; Fasciolo, J. C., and Leloir, L. F.: Am. J. M. Sc. **200**:608, 1940. (*b*) Harrison, T. R.; Grollman, A., and Williams, J. R.: Am. J. Physiol. **128**:716, 1940. (*c*) Page and Helmer.[2b]

8. Goldblatt, H.: Ann. Int. Med. **11**:69, 1937. Page.[8] Muñoz and others.[7a]

9. Kohlstaedt, K. G.; Page, I. H., and Helmer, O. M.: Am. Heart J. **19**:92, 1940.

since animals with experimental renal hypertension are reported to be hypersensitive to pitressin, tryamine,[10] carotid sinus stimuli,[11] epinephrine and perhaps to vasomotor stimuli.[12]

The behavior of the granular cells in the rabbit may offer a partial explanation for the failure to find increased numbers of these cells in clinical hypertension; of course, such a broad application may not be justifiable. In clinical hypertension, however, the kidneys are usually examined late and rarely in the early stages of the disease. If a parallel exists between the condition in the rabbit and that in man, a striking increase in granular cells would not be expected in the late stages of hypertension. In this connection it is interesting that nephrectomy coming at the later stages of clinical hypertension is often without benefit. This may be due to renal damage in the remaining kidney; but may it not likewise be due to a hypersensitivity (possibly irreversible) of the arterial musculature to small amounts of renin produced by the normal kidney or to chemical and nervous stimuli?

SUMMARY

In cellophane perinephritis the granular cells of the juxtaglomerular apparatus are increased as early as the third day after the application of cellophane to the kidney. These cells attain their maximum development in granularity, number and distribution prior to or simultaneous with the onset of hypertension and then subsequently decrease in number but probably are never reduced to normal values. It is believed that the behavior of these cells is consistent with the suggestion that they are the source of renin. It is suggested, however, that the secretions of these granular cells may render the vascular apparatus hypersensitive to pressor stimuli, which may be either chemical or nervous in nature.

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10. Verney, E. B., and Vogt, M.: Quart. J. Exper. Physiol. **28**:253, 1938. Brown, G. M.; McLean, F. J., and Maegraith, G. G.: J. Physiol. **96**:46, 1939. Ogden, E.; Brown, L. T., and Page, E. W.: Am. J. Physiol. **129**:560, 1940.

11. Heymans, C.: New England J. Med. **219**:147, 1938.

12. Dock, W.: Am. J. Physiol. **130**:1, 1940.

TERATOMA OF THE NECK IN THE NEWBORN

REPORT OF A CASE WITH AUTOPSY

FREDERIC D. CHAPMAN, M.D.

BOSTON

Congenital teratoma of the neck is relatively rare. In 1935 Pusch and Nelson [1] collected a series of 43 cases and added 1 of their own. More recently Trillat and Notter,[2] Wilson [3] and Potter [4] have reported additional cases. The case now reported is of interest because it illustrates many typical findings described by previous authors.

REPORT OF CASE

The mother of the infant was a 24 year old white woman who gave a history of one normal full term delivery three years previously. She was admitted during her second pregnancy, on Aug. 28, 1939, because of ruptured membranes; up to the time of admission pregnancy had been uneventful. Her last menstrual period was Dec. 25, 1938, eight months previously.

At the time of this admission there was evidence of marked hydramnios and a vertex presentation. Labor was difficult, and the low forceps was applied to aid delivery.

At birth the infant, a boy, was noted to have a large tumor mass about the size of his head in the anterior region of the neck. The rest of the examination of the newborn disclosed nothing remarkable. Roentgen studies showed a tumor lying in the soft tissues of the anterior region of the neck, with evidence of pressure on the trachea. Bone within the tumor was not shown.

The child nursed poorly and was dyspneic and cyanotic. He died on the ninth day, with signs of tracheal obstruction.

Postmortem Examination.—The body was that of a markedly emaciated male infant weighing 6 pounds (2,721.5 Gm.) and measuring 46 cm. in length. There was a large tumor mass in the anterior region of the neck extending from the chin superiorly to the manubrium sterni inferiorly, and laterally to the level of each ear. The tumor was covered by skin anteriorly. Posteriorly it rested on the larynx, trachea and esophagus, partially surrounding these structures. Laterally it overhung the sternocleidomastoid muscle of each side. The overlying skin was not adherent to the tumor and was easily dissected away. The tumor itself was found to be well encapsulated except where some of the smaller muscles of the anterior cervical group were stretched over the anterior surface and were partially incorporated in the capsule. Posteriorly the tumor was firmly adherent by fibrous

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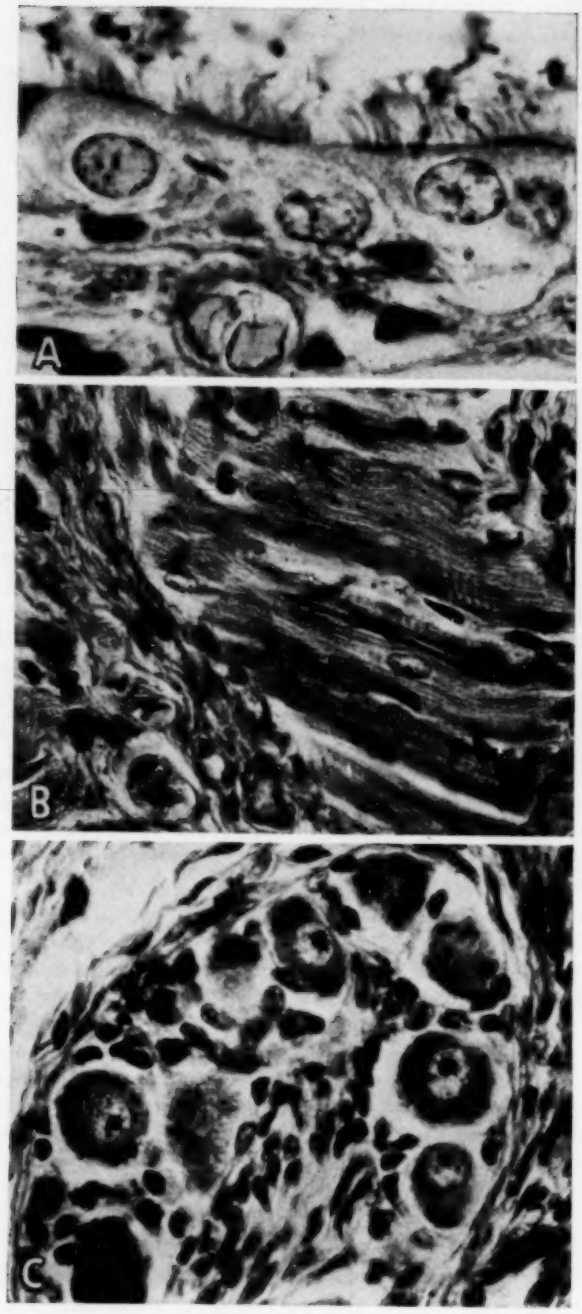
From the Mallory Institute of Pathology, Boston City Hospital.

1. Pusch, L. C., and Nelson, C. M.: Am. J. Cancer **23**:791, 1935.

2. Trillat, P., and Notter: Bull. Soc. gynéc. et d'obst. **28**:197, 1939.

3. Wilson, J. St. G.: J. Obst. & Gynaec. Brit. Emp. **46**:44, 1939.

4. Potter, E. L.: Arch. Path. **25**:689, 1938.



PLATE

(See legend on opposite page)

tissue in the midline to the hyoid bone, larynx and trachea. The thyroid gland could be identified in its normal position, lying posterior to the capsule of the tumor and compressed by the mass.

The tumor itself measured 7 cm. in vertical diameter, 11 cm. laterally and 6 cm. anteroposteriorly. It consisted of a large, roughly ovoid mass, partially cystic and partially solid, with an irregular nodular surface. The cut section revealed multiple cysts, varying in size from a few millimeters up to 3 cm. in diameter, scattered through irregular masses of firm to solid tissue. Many cavities contained clear yellow fluid, and others were filled with blood-stained to frankly hemorrhagic fluid. One large cyst, about 3 cm. in diameter, contained a firm gray polypoid mass, which filled almost the entire cavity. The mass was attached to the wall of the cyst by a narrow pedicle. The solid areas through the tumor appeared gray and showed scattered deposits of cartilaginous-like material with no orderly arrangement.

Postmortem examination of the remainder of the body showed no congenital abnormalities. The lungs showed a small amount of bronchopneumonia.

Microscopic Examination.—The types of tissue present were: fibrous connective tissue, fatty tissue, smooth muscle fibers, striated muscle fibers, cartilage, bone with bone marrow, nerve tissue showing glial cells and ganglion cells, cavernous hemangioma. The following epithelial types were noted: cuboidal, columnar, ciliated columnar, squamous, with areas of keratinization; there was also present glandular tissue showing papillary and adenomatous structures and an attempt at brain formation.

Sections taken from many different areas of the tumor showed a complex picture of the various types of tissue just outlined. Cystic cavities were seen to be lined by low cuboidal epithelium, ciliated in some areas (figure, *A*) and surrounded by young fibrous connective tissue, pieces of cartilage and hyaline connective tissue. Some of the tissue showed tubular gland structure with cylindric epithelial lining. A few bands of striated muscle were seen scattered in loose connective tissue (figure, *B*). Many cysts were lined by stratified squamous epithelium, which in some areas was heaped up and even entirely filled the lumen. Areas of hemorrhage were present, which seemed to be related to capillary cavernous sinuses. Early bone formation with bone marrow was identified. One section showed a collection of glandular acini, the appearance of which suggested a resemblance to the parotid gland or to acini of the pancreas. Another section suggested an attempt at brain formation. This tissue was lined on one side by stratified squamous epithelium, beneath which was a layer of fibrous connective tissue, and beneath the latter what appeared to be young brain substance, showing neuroglia and microglia. In another area, well formed ganglion cells were seen (figure, *C*).

EXPLANATION OF PLATE

A, lining of a cavity, showing ciliated simple cuboidal epithelium; phloxine–methylene blue stain; × 1,350. *B*, solid area of tumor, showing skeletal muscle fibers in longitudinal and transverse planes. The longitudinal fibers exhibit characteristic cross striations; phloxine–methylene blue stain; × 1,350. *C*, solid area of tumor, showing well differentiated ganglion cells; phloxine-methylene blue stain; × 1,350.

A section taken through the thyroid adjacent to tumor tissue showed the thyroid to be separated from the tumor by a thick layer of fibrous tissue. The glandular acini of the thyroid appeared somewhat flattened and compressed.

COMMENT

Many theories have been advanced in explanation of teratoma, but there is no general agreement on the subject. The main theories of genesis seek an explanation on embryologic grounds. Briefly, they include: (1) the theory of fertilization of a polar body which invades the embryo; (2) the blastomere hypothesis of Bonnet,[5] which postulates the splitting off and migration of blastomeres in the embryo; (3) the cell rest theory, based on the observed inclusion of pluripotential cells; (4) the possibility of monozygotic twinning with overgrowth of one twin; (5) the parthenogenesis of germinal cells in either normal or abnormal locations, and (6) the "organizer theory" of Spemann, as applied by Krafka,[6] which postulates an interference with the normal effect of the organizer, causing the production of secondary embryonic axes in the primitive shield, leading to the formation of a teratoma. For a more detailed discussion of this theory as a possible explanation of all forms of teratoma, the reader is referred to Krafka's [6] original article.

Russell and Kennedy [7] pointed out a few common findings associated with teratoma of the neck. Probably the most common of these is hydramnios. Another is manifest at the time of delivery of the child in the form of an abnormal presentation because the tumor prevents proper flexion of the head. Breech presentation of the child is not uncommon, and delivery usually occurs prematurely. The lesion is seen most frequently in the male infant. No hereditary influence has been determined.

In the majority of the cases reported, the infant has been dead, probably the result of dystocia caused by the tumor. However, a few cases have been reported in which the child survived delivery. Schimmelbusch [8] in 1894 reported the first case of successful removal of a teratoma of the neck with survival of the child. Swoboda [9] and Poult [10] in 1905 reported similar cures by means of surgical intervention.

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5. Bonnet, M.: Ergebn. d. Anat. u. Entwcklngsgesch. **9**:820, 1899.

6. Krafka, J., Jr.: Arch. Path. **21**:756, 1936.

7. Russell, A. W., and Kennedy, A.: J. Obst. & Gynaec. Brit. Emp. **23**:109, 1913.

8. Schimmelbusch: Klin. Wchnschr. **31**:17, 1894.

9. von Swoboda, N.: Wien. klin. Wchnschr. **9**:1062, 1896.

10. Poult, J.: Virchows Arch. f. path. Anat. **181**:101, 1905.

In the case reported here the infant was too poor a surgical risk for one to attempt surgical intervention, although the tumor was later found to be well encapsulated and would perhaps have been amenable to surgical removal.

SUMMARY

A case of congenital teratoma of the neck is reported, with the observations at autopsy. Clinically, the tumor was associated with hydramnios, premature birth and vertex presentation. The infant was a boy. The tumor was a complex teratoma, well encapsulated, and made up of many different types of mature tissue elements.

PSAMMOMATOUS DURAL ENDOTHELIOMA (MENINGIOMA) WITH PULMONARY METASTASIS

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CINCINNATI

According to Ewing,[1] metastasis from a dural endothelioma is extremely rare.

Klebs [2] stated that these tumors never metastasized, although he had observed a single case, which he referred to as one of a miliary form of endothelioma of the pia mater, in which numerous small and large gray nodules were found in the lungs. These nodules showed the same structure as the growths in the pia mater.

Lindner [3] described a tumor of the dura mater, diagnosed as endothelioma, with metastases in the urinary bladder. The patient was a 63 year old man who for several months had had sharp pains in the legs and urinary disturbances. There was a small knoblike tumor to the right of the midline below the umbilicus, together with general diminution of reflexes, ataxia and Romberg's sign. Death occurred eight months later, following a gradual downhill course. Autopsy revealed, on the anterior wall of the bladder directly above the urinary meatus, which was partly occluded, a walnut-sized soft fungiform mass. Several smaller tumors were scattered throughout the bladder. The intracranial growths consisted of soft reddish nodules just to the left of the hypophysis and a conglomeration of nodules scattered about the right middle cranial fossa. The larger nodules were adherent to the leptomeninges and pressed lightly into the brain. The falx cerebri was free of tumor, but many small nodules were present on the tentorium cerebelli. The brain was soft and pale, with areas of softening and edema. Microscopically, the neoplasm of the dura was found to consist of adenomatous-like formations of irregular polygonal cells quite densely packed, with practically no intercellular substance. The nuclei were large, pale and discrete with a distinct chromatin meshwork. Many mitotic figures were evident. Within the strands of cells, small and large round cavities or spaces were present. These spaces, some of which had a direct communication with blood vessels, were either empty or filled with the products of cell decomposition. Occasionally, the presence of isolated blood cells or small hyaline bodies was noted. The tumor of the bladder was interesting, since it showed a picture corresponding to the neoplasm of the dura mater. Many of the spaces contained nucleated red blood cells, while others contained reddish hyalin-like bodies. The diagnosis of endothelioma was quite evident.

Cushing and Eisenhardt [4] described the case of a patient with a locally recurrent meningioma, who came to autopsy after the seventeenth operation. The tissue

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From the Department of Pathology, Cincinnati General Hospital.

1. Ewing, J.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1940, p. 350.

2. Klebs, E.: Die allgemeine Pathologie, Jena, Gustav Fischer, 1889, vol. 2, p. 118.

3. Lindner, E.: Ztschr. f. Heilk. **23**:118, 1902.

4. Cushing, H., and Eisenhardt, L.: Meningiomas, Springfield, Ill., Charles C. Thomas, Publisher, 1938, p. 716.

removed at the first operation resembled leiomyomatous spindle cell sarcoma with numerous mitotic figures and areas of hyaline degeneration. Certain other areas showed a tendency to palisading of the nuclei, suggesting neuroma. Specimens removed during subsequent operations showed transformation into an epithelial-like growth, the most striking feature of which was the arrangement of the cells about a central vascular stalk. At autopsy, the most noteworthy finding was an encapsulated nodule, measuring 2.3 cm., located in the upper pole of the right lung at the bifurcation of the bronchus, and three smaller nodules, 2 to 3 mm. in diameter. Histologically, these proved to have the same structure as the intracranial growth. There were no other metastases in the body and no evidence of a possible primary growth other than that in the brain. Contrary to expectation, the parasagittal tumor removed at the last operation had not invaded the sinus or grown through the falx. Photomicrographs of this tumor showed no discernible psammoma bodies.

REPORT OF A CASE

A 72 year old Negress was admitted to the Hamilton County Home and Chronic Disease Hospital in March 1936. Her health had always been good, and noteworthy complaints were not present.

She was a well developed, somewhat overnourished woman, not acutely ill. The head and eyes were normal. Hearing was slightly diminished bilaterally. An upper denture was present. The throat, neck and thorax were normal. Throughout the lungs there were exaggerated breath sounds. Rales were not heard. The heart was not enlarged, the sounds were of fair quality, and murmurs or abnormalities of rhythm were not present. The blood pressure was 160 systolic and 85 diastolic. The abdomen showed no unusual features. The extremities and the spine were not remarkable. There was evidence of vascular sclerosis. The reflexes were physiologic. The blood Kahn test was negative. The blood count was within normal limits. The urine disclosed a trace of albumin and an occasional cell.

After a brief illness, the details of which are lacking, death occurred, in August 1937. The cause of death was not noted.

Autopsy (forty hours after death).—The body was well developed and somewhat overnourished. There was a fair amount of panniculus. The musculature was flabby and pale. The peritoneal cavity showed no changes. The pleural lining was thickened and lusterless, and several fairly dense fibrous adhesions bound the apex of the left lung to the parietal pleura. The pericardium and diaphragm were normal. The flabby heart weighed 375 Gm. The valves were somewhat thickened and slightly nodular. The myocardium showed small areas of fibrosis. The coronary vessels and aorta were thickened, with atheromatous changes. The spleen was small and flabby, the vessels prominent and the capsule thickened. The liver, pancreas and gastroenteric tract were not remarkable except for moderate postmortem change. The gallbladder was thickened and contained many spiculated stones. The kidneys were small and showed changes secondary to arteriolosclerosis. The urinary bladder, adrenal bodies, ovaries and uterine tubes presented no noteworthy changes. The uterus was the site of a soft endometrial polyp and a small fibroid.

The internal surface of the skull had a corrugated appearance due to multipie areas of hyperostosis. Firmly adherent to the dura mater on the vertex was a cauliflower-like, slightly granular, soft, brownish pink mass, measuring 5 by 2 by 3 cm. This tumor mass projected down so that it pressed on the cerebrum at the junction of the left central and longitudinal fissures, making a definite depression

in the cortex. A similar but smaller mass was found on the lateral wall of the left middle cranial fossa. The brain itself was smaller than normal and weighed 975 Gm. The arteries were atheromatous and the cortical veins congested. The leptomeninges presented no striking abnormalities with the exception of adhesions in the vicinity of the tumor. The convolutions were narrowed but flattened at their surfaces. Frontal sections of the brain after fixation revealed a well demarcated, yellowish gray, semisoft, tumor, measuring 3 by 1.5 cm., which had invaded the cerebral cortex and was located directly beneath the dural portion of the growth. The third and lateral ventricles were slightly dilated.

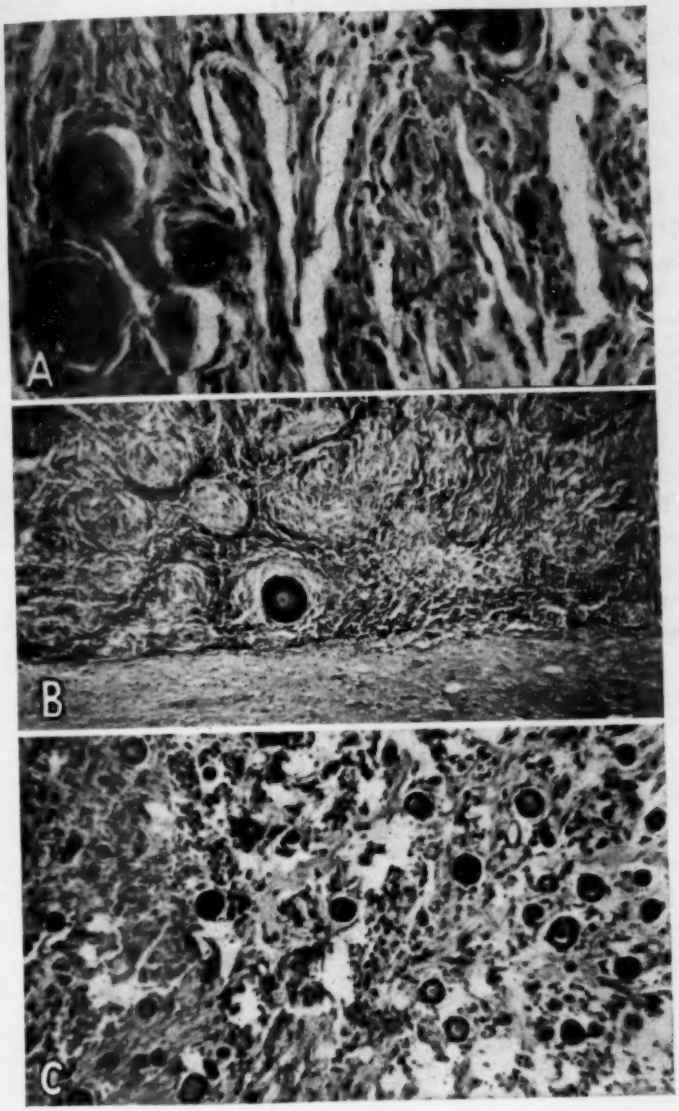
The right lung was slightly subcrepitant and weighed 550 Gm. A small nodule could be palpated in the apex and several discrete areas in the base. Sections revealed numerous patchy noncrepitant areas of lobular pneumonia. In the lower two thirds of the lower lobe there were several small, discrete, elevated brownish gray areas, varying in size from 2 to 8 mm. A single area similar to those just mentioned was also noted in the upper lobe a short distance beneath the pleura. The left lung was subcrepitant and weighed 600 Gm. Numerous patches of lobular pneumonia similar to those in the right lung were observed here. Just beneath the pleura in the base of the left lung there was a nodule measuring 2 cm. in diameter, which corresponded in character to the nodules seen in the right lung. The bronchial tree was filled with thick tenacious greenish exudate. The hilar lymh nodes were not enlarged.

Microscopic Examination.—Sections of the tumor of the dura mater revealed a moderately cellular tissue arranged in irregular dense bundles or sheaths. In some areas the stroma was arranged in whorls. Interspersed at irregular but quite frequent intervals were many concentric bodies showing various degrees of hyalinization. Many of these bodies showed calcification, and their identity as psammoma bodies was unmistakable (*A* in figure). The bundles themselves consisted of layers of polygonal and elongated spindle cells, some of which were quite flattened. The fibrous processes arising from the cells were quite dense in most parts of the tumor. The cells contained elongated or round nuclei, distinct and dark staining, some of which contained a moderately coarse chromatin network. Careful search failed to reveal any evidence of mitosis. In many portions of the growth the bundles of cells were concentrically arranged about a central lumen. In a few places actual spicules of calcium were deposited. Necrosis was not present.

The portion of the neoplasm which had invaded the brain was well circumscribed by a rudimentary, slightly vascular capsule, which showed no interruption of its continuity in the sections examined (*B* in figure). The prominent feature of the growth in the brain was the arrangement of the concentric layers of cells about a central lumen, which in most places had the appearance of a rudimentary blood vessel. Many of these vessel-like spaces contained red blood cells. A further conspicuous feature was the relative infrequency of psammoma bodies in this part of the neoplastic growth. The cerebral tissue adjacent to the tumor showed slight changes due to pressure. No invasion of blood vessels was noted.

The metastatic nodules in the lungs, although well circumscribed in the gross specimen, presented an entirely different picture when viewed under the microscope. Here infiltration had occurred, with necrosis of the involved tissue (*C* in figure). In spite of this, the most unusual feature was the relatively high degree of differentiation and maturity of the growth. The tumor corresponded in practically all respects with the dural tumor except for the delimitation. The surprising fact was that the growth was peppered with psammoma bodies. Portions of the lungs bearing no demonstrable relation to the neoplasm showed acute purulent bronchitis and lobular pneumonia with early abscess formation.

Diagnosis.—The conditions diagnosed were: acute purulent bronchitis and lobular pneumonia, with abscess formation; relatively mature psammomatous dural endothelioma (meningioma) with local invasion of the brain and metastasis to



A, section of a dural tumor (high power) showing the concentric arrangement of the cells about the psammoma bodies.

B, low power view of the portion of the tumor which invaded the cerebrum. Note the clear demarcation between the tumor and cortex.

C, the tumor as it appears in the lung (low power). Actual invasion of the lung tissue has occurred. The psammoma bodies are numerous.

the lungs; generalized arteriosclerosis; leiomyofibroma of the uterus; edometrial polyp; cholelithiasis and chronic cholecystitis; hyperostosis of the skull; post-mortem changes in the organs.

COMMENT

The clinical aspects of this case are relatively unimportant since the tumor did not produce symptoms. That it was a cause of death is not plausible, because of its small size and the fact that it had not affected vital areas. The patient undoubtedly died of acute lobular pneumonia.

The histologic picture is incontrovertibly that of a mature form of dural endothelioma of the so-called psammomatous type. Furthermore, the structure of the growth indicates that it was a well differentiated benign neoplasm. Evidence for the possibility that it was a sarcoma is wanting. In spite of the benign appearance of the primary growth, it invaded the brain and metastasized to the lungs. One cannot overlook the possibility that two tumors of similar structure arose at separate points. This conjecture is highly improbable for two reasons: 1. The histologic appearance of the tumor in the lungs is practically identical with that of the growth in the dura. 2. A primary neoplasm of this type has not been observed in the lungs. The fact that the tumor was found in the lungs leads one to the conclusion that it was carried there via the blood stream.

The 3 cases described in the literature were undoubtedly instances of endothelioma, but the growths were not as clearly differentiated or as mature histologically as that in the case now presented.

HEMOLYTIC REACTIONS FOLLOWING TRANSFUSIONS OF BLOOD OF THE HOMOLOGOUS GROUP

II. FURTHER OBSERVATIONS ON THE ROLE OF PROPERTY Rh, PARTICULARLY IN CASES WITHOUT DEMONSTRABLE ISOANTIBODIES

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BROOKLYN

In a previous paper[1] 4 cases were described in which hemolytic reactions followed transfusions of apparently compatible blood. One of the patients, who belonged to group O, had received repeated transfusions from group O blood donors, while the other 3 patients, all of group A, had been given repeated transfusions of group A blood. The patient of group O and 2 of those of group A were studied in detail in order to ascertain the cause of the reactions. The mechanism was found to be the same in all 3 patients; i. e., the reactions were due to differences between the bloods of the patients and the bloods of the donors with respect to a particular agglutinable property of human blood cells. This property proved to be identical with the agglutinogen Rh, recently discovered in human blood by Landsteiner and Wiener,[2] with the aid of an immune serum prepared by immunizing rabbits with the blood of rhesus monkeys (Macacus mulatta).

The property Rh is distributed among human beings independently of the blood groups. Approximately 85 per cent of all persons have this agglutinogen in their blood cells, while about 15 per cent lack it. Isoantibodies for Rh, unlike those for A and B, do not normally occur in human plasma, and the transfusion of blood containing Rh into patients whose blood does not have isoantibodies for this agglutinogen is ordinarily uneventful, the transfused cells surviving and functioning in

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1. Wiener, A. S., and Peters, H. R.: Ann. Int. Med. **13**:2306, 1940.

2. Landsteiner, K., and Wiener, A. S.: Proc. Soc. Exper. Biol. & Med. **43**: 223, 1940.

the patient's circulation for periods of up to three or four months.[3] However, for a certain percentage of patients who do not have Rh in their blood cells only blood that is free from Rh is suitable in the event that a transfusion becomes necessary. When blood positive for Rh is injected into such patients, the body seems to recognize the blood as foreign and eliminates it. At the first transfusion the hemolysis may be so gradual that there are no untoward symptoms and the occurrence of hemolysis is not suspected. However, this inapparent hemolysis may be followed by the appearance of Rh isoantibodies in the patient's body, and a subsequent transfusion of Rh-positive blood may result in a severe or fatal reaction. This was shown to be the sequence of events in the 3 cases previously reported,[1] the final proof being the demonstration of anti-Rh agglutinins in the serum of all 3 patients.

By using the Rh-agglutinating serum obtained from these patients for testing the bloods of a series of donors, I have been able to compile a list of standard Rh-negative and Rh-positive bloods. With the aid of standard samples of blood from these donors it has been possible to study additional cases of intragroup hemolytic transfusion reactions, and the newer results have confirmed the original impression that the factor Rh plays a major role in such reactions. Of particular interest among the newer cases encountered were some in which no isoantibodies could be demonstrated in the patients' serums, yet it was possible to demonstrate that the hemolysis was caused by Rh isoantibodies. The purpose of this second paper is to describe a number of the more recently encountered cases of intragroup hemolysis and to present some additional conclusions, based on the newer observations.

For convenience, in the present paper the cases of intragroup hemolysis have been divided arbitrarily into two classes, namely, those in which isoantibodies giving reactions unrelated to the blood groups can be demonstrated in the patients' serums, and those in which no such isoantibodies are detectable. In practice the division between these two classes is not a sharp one, a good deal depending on the sensitiveness of the technic used when testing for the isoantibodies.

CASES IN WHICH CLEARLY DEMONSTRABLE ISOANTIBODIES WERE FOUND IN THE PATIENTS' SERUMS

The case to be described first will serve to demonstrate that hemolytic reactions are not always accompanied by severe symptoms. It will also illustrate the technic used for establishing the occurrence of hemolysis in the absence of noticeable clinical symptoms.

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3. Wiener, A. S., and Schaefer, G.: M. Clin. North America **24**:705, 1940.

CASE 1.—An adult man was operated on at Bellevue Hospital, where a large calculus was removed from the pelvis of one kidney. On Sept. 12, 1940, he was given a transfusion of 500 cc. of stored compatible group A blood. There was no reaction following the transfusion. A second transfusion, five days later (September 17), was likewise uneventful. A third transfusion, six days later (September 23), was discontinued after 350 cc. of blood had been given because of the occurrence of a chill and a rise in temperature to 100 F. A blood sample obtained from the patient on September 25 and blood samples from the 3 donors were submitted to me for examination in order to ascertain the nature of this reaction.

The reaction was so mild, there being no untoward sequelae, that one might have been inclined to disregard it as being due to nonspecific and relatively innocuous agents, such as disintegrated red cells, leukocytes or platelets in the stored blood, to the presence of minute clots in the blood or to the use of improperly cleansed apparatus. However, when the serum of the patient was separated from the clot, it was found to have a slight but distinct icteric tint. Moreover, with the aid of the M,N tests it was possible to demonstrate that hemolysis of the blood of at least 2 of the 3 donors had occurred. The grouping tests revealed:

Blood	Group	Type
Patient's	A	M
1st donor's	A	MN
2d donor's	A	N
3d donor's	A	M

Since the patient's blood belongs to type M, the first donor's to type MN, the second donor's to type N and the third donor's to type M, at the time of the tests on September 25 the patient's blood was expected to give only partial agglutination in the anti-M serum (since the blood from the second transfusion does not agglutinate in the anti-M serum), and in the tests with anti-N serum, instead of no clumping, partial agglutination, due to the transfused MN and N cells. Actually, the patient's blood behaved like a normal type M blood, so that all the type N and type MN blood must have been eliminated from his circulation. Therefore, the first and second transfusions, though symptomless, had been followed by inapparent hemolysis of the donor's blood. Concerning the third transfusion no conclusion is possible on the basis of the M,N tests, as this donor belonged to type M, the same type as the patient. However, that the chill following this transfusion was associated with hemolysis was indicated by the icteric tint of the patient's serum, and was rendered probable by the evidence just presented that hemolysis had occurred after the first two transfusions.

Because the reactions occurred in a patient of group A, one might be inclined to ascribe them to differences between the patient and the donors with respect to the subgroups. While it has been shown that

transfusions of A_1 blood into patients whose blood is A_2 can stimulate the production of immune a_1 isoagglutinins,[4] this appears to be a rare phenomenon. More common in my experience is the occurrence of isoimmunization with respect to factor Rh, and this proved to be the explanation in the present case. Tests were made with the patient's serum, separated from a blood sample taken October 1 (six days after the reaction), against a series of blood samples, the reactions of which in Rh serum had been ascertained previously. Two drops of serum were mixed with a suspension of the washed blood cells (a concentration of 2 per cent in terms of blood sediment) in small test tubes, and these were centrifuged at low speed in cups containing ice water, after

Comparison of the Reactions of the Blood Serum of the Patient in Case 1, with Those of the Serums in the Three Cases of Wiener and Peters [1] and Those of an Antirhesus Immune Serum (Combined Results of Several Experiments)

Specimen	Group and Type	Reactions with Serum of Patient in Case 1 (Group A)	Reactions with Serums in Cases Reported by Wiener and Peters			Reaction with Antirhesus Serum
			Case 1 Group O	Case 2 Group A	Case 4 Group A	
1	OMN	+±	+++	..	++±	Positive
2	ON	++	++±	..	..	Positive
3	OMN	0	0	0	0	Negative
4	OMN	++±	+++	+++	..	.....
5	A_2MN	++	..	++±	++	.....
6	A_1M	++	..	++	++	.....
7	OM	0	..	0	..	Negative
8	OM	++	++±	..	..	Positive
9	A_1MN	++±	..	+++	..	.....
10	A_1MN	++	..	++±	..	.....
11	OMN	0	..	..	0	.....

The intensity of the agglutination is indicated by the number of pluses, +++ being the maximum reaction possible, namely, clumping of the cells into a single large mass. A zero indicates absence of agglutination, and blank spaces (..) indicate that a test was not made.

they had first stood for several minutes in the ice water. The tubes were then placed in a rack, which was gently shaken, and the reactions were read both macroscopically and microscopically. By this technic (Wiener and Peters [1]) it was demonstrated that besides the isoagglutinin β, the patient's serum contained an isoagglutinin giving reactions unrelated to the blood groups but corresponding to property Rh (table). This completed the proof that despite the mild and apparently innocuous nature of the reaction following the third transfusion in this patient the chill was symptomatic of the occurrence of a hemolytic reaction based on differences with respect to the factor Rh.

4. Wiener, A. S.: Subdivisions of Group A and Group AB: II. Isoimmunization of A_2 Individuals Against A_1 Blood, with Special Reference to the Rôle of the Subgroups in Transfusion Reactions, J. Immunol., to be published.

The patient was given no further transfusions as his convalescence was satisfactory, and he was discharged from the hospital in relatively good health.

The present case demonstrates, in conformity with those previously described,[1] that in patients having the capacity to form anti-Rh isoantibodies the donor's blood, if it contains Rh, may be destroyed even after the first transfusion. However, this is not associated with any untoward symptoms at first, because the process is a gradual one. As the transfusions are repeated, at properly spaced intervals (five to seven days or longer) the reactions tend to become more severe, and if the transfusions are continued, a severe or fatal reaction finally results. In the present case, fortunately, the nature of the reactions was recognized before harm had resulted.

These observations also suggest the possibility of recognizing the occurrence of inapparent hemolysis and thus anticipating and possibly preventing severe hemolytic reactions in instances in which further transfusions are required. If no significant rise in hemoglobin occurs, blood samples from patient and donor should be typed for M,N; if the patient and donor bloods are of different types with respect to M,N, the occurrence of hemolysis of the donor's cells can be readily established as already described. If the bloods are of the same M,N types, one may have to rely for the diagnosis on a high icteric index of the patient's serum or on the demonstration of irregular isoagglutinins in the serum. To illustrate these points the following case is described:

CASE 2.—A Negro girl was admitted to Mercy Hospital, Baltimore, Dec. 16, 1940, complaining of abdominal pain. At operation a retrocecal ruptured appendix was found, with localized peritonitis. December 23 the patient, whose blood belongs to group B, was given a transfusion of 250 cc. of compatible group B blood, and on the following day, another 250 cc. from the same donor. Four days later the patient was given 250 cc. of group B blood from a second donor. The following day, the direct matching test, set up by the technician prior to giving a final 250 cc. of blood from the second donor, showed clumping of the donor's cells by the patient's serum at ice box temperature. Repetition of the matching on January 4 again showed clumping in the combination of patient's serum with donor's cells, but not in tests of the patient's serum against the patient's own cells. The bloods were then referred to me by Sister Celeste for further study.

The grouping tests on the bloods of the patient and the donors revealed:

Blood	Group	Type
Patient's	B	MN
1st donor's	B	MN
2d donor's	B	M

Since the first donor belongs to the same type as the patient, the fate of his blood in the patient's circulation could not be ascertained with the M,N tests. At any rate, the reactions given by the patient's blood demonstrated that the 250 cc. of blood received from the second donor (type M) had been eliminated from the patient's (type MN) circulation. Moreover, the presence of a weak but definite irregular isoagglutinin in the patient's serum could be demonstrated, confirming the technician's previous observations. Unlike the post-transfusion serums previously described,[1] this patient's serum gave rather indistinct reactions at low temperatures and clearer (though weak) reactions at higher temperatures, being similar in its behavior to the serums recently described by Levine, Katzin and Burnham.[5] The reactions were best brought out by the technic suggested by Levine, namely, by incubating the tubes in a water bath at 37 C. for ten to fifteen minutes and then centrifuging at low speed at room temperature. Four bloods (1 Rh negative and 3 Rh positive) were tested with the patient's serum, and the reactions were found to agree with those of a standard Rh antiserum. In addition, the patient's blood cells were found to be Rh negative. Though the series of bloods tested in this case was small, the evidence presented indicates that the inapparent hemolysis of the second donor's blood was caused by individual differences between the bloods of patient and donor with respect to property Rh, and, most likely, the alertness of the technician served to prevent the occurrence of a severe hemolytic reaction.

In the 2 cases just described, the existence of an intragroup incompatibility was discovered before any harm had resulted, and the outcome was a happy one in both instances. In the 2 cases now to be described, the existence of intragroup incompatibility was responsible for severe hemolytic reactions, one of which terminated with the death of the patient.

CASE 3.—A physician's wife was admitted to the Adelphi Hospital, Brooklyn, on July 8, 1940, with a diagnosis of post-transfusion uremia. The patient had 3 normal children, 6 years, 4 years and 2 years old, respectively, but the husband stated that each pregnancy had been complicated by toxemia and convulsions. The present illness began with the fourth pregnancy, also complicated by toxemia. This pregnancy terminated spontaneously at three and one-half months, with extrusion of blood clots and fetal tissue from the vagina. Because of the loss of blood, a blood count was made on July 3, which revealed a red cell count of 3,000,000, with hemoglobin 60 per cent. The patient was given a direct transfusion of 500 cc. of compatible group O blood from her aunt, this being the first transfusion she had ever received. (The husband was not used

5. Levine, P.; Katzin, E. M., and Burnham, L.: Proc. Soc. Exper. Biol. & Med. **45**:346, 1940.

because his blood belongs to group A.) Shortly after the termination of the transfusion the patient had a severe chill and a high temperature and became noticeably jaundiced. The bloods of patient and donor were then retested, and the original finding that both belonged to group O confirmed. The clinician therefore ascribed the chill, fever and jaundice to infection, and the patient was given sulfanilamide. The patient felt fairly well after the transfusion, but on July 5 her hemoglobin was only 44 per cent and the red cell count 1,970,000. On July 6, 250 cc. additional blood was taken from the patient's aunt and administered to the patient by the citrate-gravity method. After this transfusion it was noticed that the patient ceased to void and became drowsy and jaundiced. A clinical examination of her blood on July 10 revealed urea nitrogen to be 150 mg. and creatinine 4.5 mg. per hundred cubic centimeters. On July 11 the blood urea nitrogen reached 165 mg. and the blood creatinine 5 mg. per hundred cubic centimeters. On July 12 Dr. Ravich performed a bilateral decapsulation of the kidneys, the operation being followed by intravenous administration of 500 cc. of blood plasma, followed by 2,000 cc. of Ringer's solution, plasma being given instead of whole blood since it was now clear that the blood transfusions were responsible for the uremia. Diuresis set in promptly after the operation, and the patient's general condition improved. I was called to see the patient for the first time on July 15, three days after the operation, to determine the cause of the transfusion reactions, and whether or not more blood could be transfused safely. As is pointed out later, it was possible to prove that the hemolytic reactions were due to intragroup blood differences between the patient and the donor with respect to factor Rh. The patient's husband was then assured that a transfusion of Rh-negative group O blood would be safe, but he decided to take his chances without further transfusions even though the patient's hemoglobin was only 30 per cent. Fortunately, the patient convalesced without incident and when seen again on August 5 had completely recovered, aside from anemia with 55 per cent hemoglobin.

In this case, in contrast to cases 1 and 2, a severe hemolytic reaction followed the very first blood transfusion. A plausible explanation for such reactions was proposed by Levine and Stetson [6] when they attempted to explain a transfusion reaction observed by them. According to these authors, their patient was immunized against an antigen unrelated to the blood properties A and B, which was present in the blood of the fetus but lacking from the patient's own blood. The fetus is assumed to inherit the factor from its father. Wiener and Peters [1] showed that the intragroup hemolytic reactions thus far reported, in general, fall into one of the following two categories: (1) those in patients receiving repeated transfusions, the initial transfusion providing the stimulus for the production of the isoantibodies responsible for the hemolytic reaction; (2) those in patients who had never previously received a blood transfusion. The fact that every hemolytic reaction reported which belongs to the second category occurred in a woman who had recently given birth or had had a miscarriage or a stillbirth lends

6. Levine, P., and Stetson, R. E.: J. A. M. A. **113**:126, 1939.

support to the idea that the fetus is the source of the antigen which stimulates the formation of the antibodies in such patients.

In case 3 the blood-grouping tests on patient and donor revealed the following:

Blood	Group	Type	Rh Reaction
Patient's	O	M	Negative
Donor's	O	MN	Positive

Despite the fact that the patient had received the transfusions less than two weeks before these tests were made, the patient's blood reacted like a typical M, so that none of the donor's type MN blood remained in her circulation. This confirmed the conclusion that a hemolytic transfusion reaction had occurred. By the cold centrifuge technic (Wiener-Peters[1]) clumping was observed in the mixture of the patient's serum with the donor's cells, but no agglutination occurred in the mixture of the patient's serum with her own cells.[7] Moreover, tests with an anti-Rh immune serum showed the patient's blood to be Rh negative and the donor's blood to be Rh positive. Finally, a series of 16 bloods were tested with the patient's serum in parallel with an anti-Rh immune serum, the reactions with the two serums exhibiting satisfactory agreement, although the patient's serum did not give as distinct reactions as the serum in case 1 or those previously described.[1] This proved that the hemolytic reaction in this case was due to the difference between the bloods of patient and donor with respect to factor Rh.

The blood of the patient's husband and the blood of one of the children were tested with the following interesting results:

Blood	Group	Type	Rh Reaction
Patient's	O	M	Negative
Husband's	A	M	Positive
Son's	O	M	Positive

The findings in this family, as well as unpublished observations of Landsteiner and Wiener which indicate that the property Rh is inherited as a simple mendelian dominant, supply further evidence in support of the theory of isoimmunization of the mother by the fetus in utero. It is of interest to note that Levine, Katzin and Burnham[8] have compiled a series of cases of intragroup hemolytic transfusion reactions in postpartum patients. Eleven such patients with demonstrable isoantibodies in their serums were studied, and the reactions of 8 of the serums were found to correspond to property Rh. These observations, as well as those in case 3 just described, fulfil the prediction made by Wiener and

7. Cross-matching tests by the common slide technic were negative.

8. Levine, P.; Katzin, E. M., and Burnham, L.: J. A. M. A. **116**:825, 1941. Levine, P., and Katzin, E. M.: Proc. Soc. Exper. Biol. & Med. **45**:343, 1940.

Peters that "an Rh negative woman carrying an Rh positive fetus might react by producing Rh isoantibodies." Thus, agglutinogen Rh can act as an antigen in this manner as well as by transfusion.

In the case now to be described the patient's death followed an intragroup hemolytic transfusion reaction caused by factor Rh, and it is of interest because of certain of its clinical features and a successful transfusion of Rh-negative blood, though this was too late to prevent the demise of the patient.

CASE 4.—An adult white man was admitted, Oct. 10, 1940, to the service of Dr. W. S. Tillet at Bellevue Hospital, with the diagnosis of possible hyperparathyroidism. His chief complaint on admission was pain in the bones of his back and hip. During the previous six months he had been at two other institutions, where generalized osteoporosis was found by roentgen examination and the diagnosis of multiple myeloma was suggested. However, the urine did not show Bence Jones protein, and puncture of bone marrow revealed proliferation of what were thought to be the myeloid cells. At one of the institutions the patient was given three transfusions of group B bank blood. The first, March 14, 1940, was followed by a rise in temperature to 101 F. and an urticarial rash; the second, March 29, resulted in a rise of temperature to 101.6 F., but no chill; a third, April 16, was entirely symptomless. The blood calcium on admission was 12.5 mg. per hundred cubic centimeters, but at operation no parathyroid tumor was found. The postoperative condition was good except for moderate anemia (hemoglobin, 65 per cent; red blood cell count, 3,590,000).

October 23 the patient, who belonged to group B, was given his first transfusion at Bellevue Hospital of apparently compatible group B bank blood. This had to be discontinued after 300 cc. had been administered, because of the occurrence of a severe chill, nausea, vomiting, and a rise in temperature from 100 F. to 103 F. Eight days later the hemoglobin was 65 per cent and the red cell count 3,540,000. By December 4 the red cell count had dropped to 2,340,000 and the hemoglobin to 55 per cent. (The blood at this time contained 14.3 mg. of calcium and 3.1 of phosphorus per hundred cubic centimeters.) A second transfusion, this time of citrated fresh blood, was started on December 6 but had to be discontinued because of pain in the back, a moderate chill, a rise in temperature to 103 F. and a rise in the pulse rate to 144. December 10, after a careful cross-matching test, a third transfusion of apparently compatible blood of group B was started by the drip method, but this had to be discontinued after 250 cc. had been given because of a severe chill. To treat the reaction, an injection of solution of epinephrine hydrochloride U. S. P. was given, followed by administration of sodium bicarbonate. Following the third transfusion, the anemia became more severe; December 18 the hemoglobin was only 40 per cent and the red cell count 1,390,000. December 22 a fourth transfusion, this time of 500 cc. of apparently compatible group B blood, was given by the direct syringe-valve method. One-half hour after the completion of this transfusion the patient had a severe shaking chill and an urticarial eruption appeared on the skin. He was given an injection of solution of epinephrine hydrochloride U. S. P., which controlled the reaction. The following day the patient was noticeably jaundiced and his temperature was 103.8 F. There was no anuria, but the urine was cherry red, and the benzidine test was strongly positive. By December 25 the hemoglobin had dropped to 21 per cent and the red cell count to 1,040,000. The following day the hemoglobin was 18 per cent, and the patient was still

definitely jaundiced. At this point I was consulted concerning the possibility of giving further transfusions without the occurrence of a hemolytic reaction.

The resemblance between the clinical history in this case and those in the cases described by Wiener and Peters [1] indicated that we were dealing with a hemolytic transfusion reaction caused by the presence in the patient's body of isoantibodies giving reactions unrelated to the blood group. On the basis of probabilities it was predicted that the factor responsible might prove to be Rh. As the patient when first seen was in a desperate state (he was markedly dyspneic, probably because of the severe anemia) an immediate transfusion of Rh-negative blood was advised. A donor with Rh-negative group B blood was not available, so a donor with Rh-negative group O blood was selected. By the time the transfusion was started, the evening of December 27, the patient was moribund, his hemoglobin having dropped to 12 per cent. Following the transfusion, there was no untoward reaction of any sort, but the patient died two hours later, undoubtedly because of the irreversible damage of the tissues caused by the anemia. However, it was possible, as is pointed out in the following paragraphs, to demonstrate by tests on antemortem and postmortem blood samples that the Rh-negative group O blood administered at the last transfusion was compatible, in contrast to the blood given at the other four transfusions.

Tests made four days after the fourth transfusion corroborated the grouping of the patient's blood as group B. Moreover, while specimens of the bloods of the donors were not available for further tests, it was found that the patient belonged to type N. As blood donors are selected only for their blood group, not their M,N type, and as only 1 of 5 persons belongs to type N, the chance that all 4 donors whose blood was used in the first four transfusions belonged to type N, i. e., the same type as the patient, is only $(\frac{1}{5})^4$ or about 1 in 625. It is reasonable to assume, therefore, that not all the donors belonged to type N, but that one or more belonged to type M and type MN. However, the patient's blood after the fourth transfusion reacted like a normal type N blood, so that any type M or type MN blood he had received had been eliminated from his body. This supports the clinical impression that the patient's symptoms were caused by hemolytic transfusion reactions.

The serum obtained from a sample of blood drawn from the patient before the last transfusion was found to contain, in addition to the regular isoagglutinin α, an isoagglutinin giving reactions unrelated to the blood groups. This isoagglutinin differed in certain important respects from those described in cases 1 and 3 of this paper and in the paper of Wiener and Peters.[1] First of all, the reactions at low temperature were indistinct, while tests at room or body temperature gave cleancut results. The most satisfactory results were obtained by mixing 2 or 3 drops of the patient's serum with 1 drop of the blood cell sus-

pension being tested (concentration, 2 per cent in terms of blood sediment) in a small test tube, incubating the mixture at 37 C. in a water bath for five minutes, then centrifuging at low speed at room temperature. When the mixtures were gently shaken, the sediment either broke up completely, so that under the microscope each red cell was separate and distinct, or the sediment remained clumped in large macroscopic masses of agglutinated cells.[9] The irregular isoagglutinin present in the patient's serum was obviously related to the hemolytic reaction.

In order to determine whether or not the isoantibody in the patient's serum was an Rh agglutinin, a random series of blood samples from group O and group B persons was tested. Among 68 subjects 20 were found whose blood failed to agglutinate in the patient's serum, so that the incidence of negatively reacting bloods was 29.3 ± 3.3 per cent. On the other hand, among 220 persons I found 29, or 13.5 ± 1.5 per cent, whose blood failed to agglutinate in standard Rh antiserums (from patients having post-transfusion reactions). The difference in the incidence of negative reactions is 15.8 ± 3.6 per cent, and this evidence indicates that the antibody in the serum of the patient of case 4 is not an Rh agglutinin. When the reactions of the patient's serum were compared directly with those of the Rh antiserums, however, a marked correlation between the reactions was observed. Thus, among 28 bloods tested with both sorts of reagents, 13 were agglutinated by both serums and 11 by neither serum, while only 4 were agglutinated (usually weakly or only moderately) by Rh antiserums but not by the patient's serum. These results indicate a possible relationship between the two sorts of antibodies, and it appears that in case 4 we may have been dealing with an Rh-like antibody and the corresponding agglutinogen. The character of the isoantibody has also been studied by Dr. K. Landsteiner, whose findings will be reported elsewhere.[10]

Since the isoantibody in the patient's serum was not a true Rh agglutinin, the possibility existed that the Rh-negative blood of the donor selected for the final transfusion may not have been compatible. However, tests made before the transfusion was given showed that the donor's cells were not agglutinated by the patient's serum even when the centrifuging technic just described was used. Further convincing evidence that the donor's blood was compatible was obtained from tests made on a sample of blood obtained from the patient post mortem. The blood used for the last transfusion belonged to group O, type MN, while the patient's blood, it will be recalled, belonged to group B, type N. The patient's blood post mortem gave partial agglutination with anti-M serums, and the usual grouping tests showed it to contain a mixture of

9. However, a few bloods gave reactions of intermediate intensity.

10. Landsteiner, K., and Wiener, A. S.: Unpublished data.

group B and group O red cells, proving that the donor's blood had not been hemolyzed. Moreover, the patient's serum post mortem still contained the irregular isoagglutinin, its reactions being of about the same intensity as those with serum obtained before the last transfusion. Had incompatible blood been transfused, the isoagglutinin should have been absorbed.[11]

Aside from its unique serologic features, the present case is of interest from the clinical standpoint. A transfusion of 500 cc. of blood in an adult should result in a rise in hemoglobin of about 8 to 12 per cent. The patient of case 4 was given four transfusions during a period of two months. Most of them had to be discontinued before the full 500 cc. was given because of the occurrence of a reaction. However, the patient did receive a total of 1,350 cc. of blood, or sufficient to produce a rise in hemoglobin of almost 30 per cent. That no rise in hemoglobin resulted is not surprising, as the blood received at each transfusion was hemolyzed. Somewhat startling, however, is the marked drop in hemoglobin and in the red cell count, particularly after the fourth transfusion. A similar, though not as pronounced, drop in hemoglobin was observed by Wiener and Peters [1] in some of their cases. It seems that repeated transfusions of incompatible blood not only give rise to hemolysis of the donor's blood cells but also initiate some mechanism that causes destruction of the patient's own blood cells.[12] As is demonstrated in case 5, this destruction of blood can be arrested, if it has not proceeded too far, by the transfusion of truly compatible blood. Accordingly, this case illustrates another mechanism which can lead to the death of the patient after transfusions of incompatible blood.

In the first 3 cases described in this paper, the incompatibility between the bloods of patients and donors was based on individual differences with respect to factor Rh. If case 4 of this report is placed in the same category and the 3 cases previously reported by Wiener and Peters are added to these, the total number of such cases described by us is raised to 7. To this number can be added a case referred to me by Scott, who is reporting the case in detail elsewhere.[13] The serum concerned in a case of postpartum hemolytic transfusion reaction observed by Levine was tested together with him by me and found to give reactions corresponding to property Rh. Levine subsequently

11. Wiener and Peters,[1] case 2.

12. A factor contributing to the drop in hemoglobin in the present case was undoubtedly the underlying disease, since postmortem examination failed to show the presence of a parathyroid tumor but instead revealed diffuse infiltration of the bone marrow by peculiar small mononucleated and binucleated cells.

13. Scott, J. E., and Conant, J. S.: Successful Transfusions Following a Previous Hemolytic Reaction Due to Rh and Anti-Rh Factors: Report of a Case, Am. J. Clin. Path., to be published.

analyzed the irregular isoagglutinins in the serums of 11 patients who had postpartum hemolytic transfusion reactions and found that in 8 (including the serum tested by me) the antibodies in question corresponded to property Rh. All these results combined give a grand total of 15 intragroup hemolytic transfusion reactions definitely known to be due to the Rh factor and 1 due possibly to an Rh-like factor. In contrast to this is the smaller number of intragroup hemolytic transfusion reactions due to properties other than Rh. Three such cases have evidently been found by Levine. I have encountered only 2 instances of isoimmunization of A_2 persons to the agglutinogen A_1 and but 1 instance of isoimmunization to agglutinogen P.[14] Moreover, not a single instance has been found to date of isoimmunization against agglutinogens M or N. These facts serve to emphasize the importance of the role played by property Rh in transfusion reactions, and, as will now be shown, can be applied when attempting to ascertain the cause of intragroup hemolytic transfusion reactions in patients in whose serum isoantibodies are not demonstrable.

CASES IN WHICH ISOANTIBODIES WERE NOT DEMONSTRABLE IN THE PATIENTS' SERUMS

DeGowin and Baldridge[15] have described 2 fatal hemolytic transfusion reactions which apparently could not be explained on the basis of in vitro tests. Two hemolytic transfusion reactions in patients whose serum was without detectable isoantibodies were also encountered by me.[16] These reactions offered a challenge, since the failure of direct matching tests to reveal the incompatibility between the bloods of patient and donor constituted an obstacle to the selection of compatible donors for the patients. More recently, armed with the experience acquired in cases in which hemolytic transfusion reactions occurred in patients whose serum contained demonstrable isoantibodies, I have been able to transfuse blood safely to a number of such patients despite the absence of demonstrable isoantibodies from their serum.

In the cases in which intragroup hemolytic transfusion reactions occurred with demonstrable isoantibodies, it was noticed that the irregular isoagglutinins in the patients' serum were, as a rule, of low potency. Moreover, the isoantibodies were present in the patients' serum only for relatively short periods after transfusions. Accordingly,

14. These were all discovered in the cross-matching tests preliminary to transfusion, rather than after the occurrence of hemolytic transfusion reactions (Wiener and Peters.[1] Wiener [4]).

15. DeGowin, E. L., and Baldridge, C. W.: Am. J. M. Sc. **188**:555, 1934.

16. One was reported by H. Mandelbaum (Ann. Int. Med. **12**:1699, 1939); the other, by Wiener and Peters[1] (case 3).

the cases in which isoantibodies are not detectable in the serum can be divided into two classes:

1. Cases in which the patient's serum contains appreciable amounts of isoantibodies, but these isoantibodies are not detected because

(*a*) Tests are made too soon after the transfusion (within the first three or four days), during the negative phase that follows injections of incompatible blood.

(*b*) Tests are made too long after the transfusion of incompatible blood, after the isoantibodies have disappeared from the patient's plasma.

(*c*) Tests are made at the appropriate time, but the technic of making the tests is not sufficiently sensitive. Thus, the use of the slide technic in place of the tube technic (with or without centrifuging) will usually fail to reveal the presence of these special isoantibodies. Moreover, if the patient's blood is sent through the mails, or not tested at once, and has not been taken properly and kept on ice until tested, the isoagglutinins may deteriorate before the tests can be carried out.

2. Cases in which a hemolytic transfusion reaction has occurred but the patient's serum at no time contains isoagglutinins detectable by the technics available. Possibly in such cases the isoantibodies remain fixed to the reticuloendothelial cells which produce them, none or only inappreciable amounts of the isoantibodies being set free in the blood stream.[17]

In cases belonging to the first category it may be possible, by using an appropriate technic, to demonstrate the isoantibodies in the patient's serum and with the aid of this finding to select donors of compatible blood. In cases belonging to the second category it will be more difficult to select donors of compatible blood; in such instances the only criterion that may be available is the result of a biologic test. However, in view of the fact that the majority of hemolytic reactions with demonstrable isoantibodies have been found to be due to the Rh factor, it seems reasonable to assume that a similar situation holds in cases in which the antibodies are not demonstrable. Accordingly, when transfusions are required and previous transfusions have caused untoward symptoms, it may be worth while to try cautiously transfusions of Rh-negative blood. This procedure was followed in the case now to be described, with gratifying results.

Case 5.—An Italian white girl, 2½ years of age, was admitted to the Jewish Hospital of Brooklyn, service of Dr. B. Kramer, Nov. 9, 1940, with a history of fever, cough and sudden pallor of one day's duration. The patient had had one

17. The situation here is probably analogous to that which obtains in tuberculin sensitivity.

previous admission to the hospital, January 15, on account of rapidly developing anemia following sulfanilamide therapy for tonsillitis. At the first admission the hemoglobin was only 34 per cent, but after a transfusion of 200 cc. of compatible, citrated group B blood the hemoglobin rose, two days later (January 17), to 65 per cent and January 22, when she was discharged from the hospital, to 81 per cent.

At the subsequent admission she had a temperature of 103 F., a hemoglobin content of 44 per cent and a red cell count of 2,340,000. The nature of the anemia became clear when a smear of the splenic pulp was found to contain numerous sickle cells. November 12 the patient was given a transfusion of 175 cc. of citrated group B blood, and the next day, 100 cc. of blood from the same donor. Neither transfusion was followed by a febrile reaction, and November 14 the hemoglobin had risen to 57 per cent and the red cell count to 3,340,000. By November 18, however, the hemoglobin had dropped back to 53 per cent. Two days later 200 cc. of blood from a second group B donor was transfused. There was no immediate reaction, but the same evening the temperature rose from 99 F. to 103 F., and the next day the patient became noticeably icteric and her spleen and liver were considerably larger and firmer. One week later, November 27, another 200 cc. of blood was transfused from a third group B donor. There was no noticeable reaction to this transfusion, but the next day the patient appeared more icteric and the spleen became larger. The hemoglobin remained at 50 per cent. Two days later 250 cc. of blood was transfused from the same donor. Again there was no immediate reaction, but eight hours later the temperature rose to 102 F., the hemoglobin dropped to 28 per cent and the patient's jaundice deepened.

Up to this point the patient's unfavorable responses to the blood transfusions had been blamed on the underlying disease (sickle cell anemia), and when the patient's condition became critical after the transfusion from the third donor, some of the clinicians urged that splenectomy be performed immediately. Others felt, however, that the transfusions might be responsible for the symptoms, and I was requested to see the patient. On the basis of the history, the diagnosis of hemolytic anemia due to repeated blood transfusions and intragroup incompatibility seemed indicated. To exclude the possibility that the hemolysis was due to the underlying disease, however, it was first necessary to show that the hemolysis was specifically directed against the donors' bloods rather than that the bloods of the donors and patient shared equally in the hemolytic process. This was established with the aid of the M,N tests, examination of the bloods of the patient and donors revealing the following:

Blood	Group	Type
Patient's	B	N
1st donor's	B	MN
2d donor's	B	MN
3d donor's	B	N

While no definite statement can be made regarding the third donor, who belongs to the same type as the patient (type N), the first and second

donors belong to type MN, and as the patient's blood behaved like a normal type N blood, all the blood received from these two donors must have been eliminated from the patient's circulation. In view of these findings and the clinical symptoms following the transfusions from the third donor it seems reasonable to conclude that the blood of the third donor as well had been incompatible and was hemolyzed.

These results established that we were dealing with hemolysis caused by repeated blood transfusions. It then became necessary to determine the nature of the intragroup incompatibility, since the patient was urgently in need of transfusions of compatible blood. As only two days had elapsed since the last transfusion, it is not surprising that the search for irregular isoagglutinins in the patient's serum was fruitless. No serum was available for testing bloods of group B for the Rh agglutinogen, but for the reasons already given I guessed that the hemolytic reaction was probably due to this factor and advised a transfusion of Rh-negative blood. A donor of Rh-negative group B blood was not available, so a donor of Rh-negative group O blood was selected and 100 cc. of his blood transfused to the patient. There was no untoward reaction of any sort, and the patient seemed brighter after the transfusion. Moreover, on the following day it was shown with the ordinary blood grouping as well as the M,N tests that the donor's blood had not been hemolyzed. (The donor belonged to group O, type MN; the patient, it will be recalled, to group B, type N.) A second transfusion of 100 cc. from the same donor was equally successful, and the patient's hemoglobin rose to 45 per cent. More blood was needed by the patient, and to make the demonstration more convincing a different donor of Rh-negative blood (this one belonged to group O, type M) was used for the next two transfusions of 100 cc. each. There was no reaction to the transfusions from the second donor, the blood-grouping and M,N tests again being used to demonstrate that the donor's blood was not hemolyzed. Moreover, the patient's clinical condition improved markedly; her hemoglobin rose to 75 per cent and remained at that level until her discharge from the hospital on December 16. When the patient was again seen by me one month later, she was in good health, and her hemoglobin was 72 per cent.

For clinicians the most convincing aspect of the proof that in case 5 we were dealing with hemolytic transfusion reactions caused by Rh isoantibodies was the patient's excellent response to only 400 cc. of Rh-negative blood (although she belonged to group B and the Rh-negative donors to group O), while transfusions of more than a liter of group B blood taken without regard to the Rh factor (though apparently compatible by the in vitro tests) not only failed to help the patient but seemed to aggravate her condition. The patient weighed 25 pounds (11 Kg.), and therefore had a blood volume of about 900 cc. The liter of

group B blood, if really compatible, should have caused a rise in hemoglobin of more than 100 per cent; actually, the hemoglobin dropped from 44 to 27 per cent. On the other hand, the 400 cc. of Rh-negative blood resulted in almost the precise rise in hemoglobin that was expected by calculation, namely, from 27 to 75 per cent. The fact that this blood was intentionally taken from two different Rh-negative donors made the demonstration even more convincing.

As has already been pointed out, the initial tests made on the patient's serum for immune isoantibodies were made only two days after the transfusions from the third donor. As the patient was in the "negative phase," failure to detect isoantibodies at that time was not unexpected. However, although the tests for isoantibodies were repeated daily or at intervals of a few days during the remainder of the patient's stay in the hospital, at no time could such antibodies be demonstrated, either by the slide technic or the centrifuge tube technic at room, refrigerator or body temperature. Accordingly, case 5 is an example of the occurrence of hemolytic transfusion reactions without demonstration of isoantibodies in the patient's serum. Yet, as has already been pointed out, it was possible to obtain compatible donors by assuming that the factor Rh was responsible for the reactions. While no standard Rh antiserum was available for testing the bloods of the patient and donors, it was possible to test these bloods later on with the serum of the patient of case 4, which contained Rh-like antibodies. It was found that while the patient's blood was not agglutinated by this serum, the bloods of the second and third donors were strongly agglutinated, while the blood of the first group B donor gave only weak or negative reactions. This lends additional support to the conclusion that the incompatibility in case 5 involves property Rh and explains in part the initial satisfactory clinical response to the transfusion from the first group B donor. Incidentally, an attempt was made to demonstrate the patient's sensitivity to Rh-positive blood by a skin test, but intracutaneous and subcutaneous injections of the Rh-positive blood both failed to elicit local skin reactions.

The role played by the transfusion given at the first admission is uncertain, since that donor's blood was not available for tests for the Rh factor. The satisfactory clinical response to that transfusion could be explained by assuming that the donor's blood, by chance, did not contain this factor. Another possibility is that the patient had not yet been sensitized to the Rh agglutinogen. If it can be assumed that the donor's blood actually contained Rh, the transfusion given during the first stay in the hospital may have been the sensitizing one.

Case 5 demonstrates that in instances of intragroup hemolytic transfusion reactions in which the patient's serum does not contain detectable isoantibodies, the most reasonable assumption is that the reaction is due

to the Rh factor. The reactions in cases 6 and 7 are examples of this type of transfusion reaction in postpartum patients.

CASE 6.—A 35 year old woman was first seen Oct. 7, 1940, with a chief complaint of anuria, which had been present for five days. She had been admitted to the hospital one week previously, while in labor, with the following history: Her first gestation occurred in 1927 and terminated with the birth of a normal infant though three weeks premature. In 1929 she had a miscarriage, and in 1932 she delivered a normal full term infant. During her present pregnancy she had toxemia; her blood pressure was 180 systolic and 120 diastolic. At the onset of labor the fetal heart beat was audible, but during labor the blood pressure rose to 214 systolic and 136 diastolic, there was a premature separation of the placenta and a stillbirth resulted. Immediately after the delivery the patient was given a transfusion of 500 cc. of fresh, citrated, apparently compatible group O blood, the husband acting as donor. There was no immediate reaction, but following the transfusion complete anuria set in. When I first saw the patient, the blood urea nitrogen had risen to 95 mg. per hundred cubic centimeters. Grouping tests on the bloods of the patient and her husband revealed:

Blood	Group	Type	Rh Reaction
Patient's	O	MN	Negative
Husband's	O	MN	Positive

While direct matching tests between the patient's serum and donor's cells by the centrifuge technic at room, refrigerator and incubator temperatures were negative, the evidence pointed to a hemolytic transfusion reaction due to the Rh agglutinogen. At the time these tests were made the patient was drowsy (due to the uremia), her hemoglobin was only 13 per cent and a transfusion was urgently needed. I suggested waiting until a donor of compatible blood could be found, but apparently the transfusionist was not advised of the situation and proceeded with another transfusion of 500 cc. of group O blood. This was followed by a severe chill, and the following day the patient was noticeably jaundiced. Two transfusions of 500 cc. each from two different donors of Rh-negative group O blood, selected by me, were then given. These transfusions were not followed by any reactions, and the patient's hemoglobin rose to 40 per cent. However, the anuria persisted, and the patient died a few days later of uremia.

Case 6 is an instance of post-transfusion hemolysis due to the Rh factor in a patient whose serum was without demonstrable isoantibodies, the fetus in utero being the source of the foreign antigen which immunized the patient. It is noteworthy that a transfusion from a group O donor, selected at random, was followed by a chill and icterus, while transfusions from two different selected Rh-negative group O donors were uneventful and resulted in approximately the expected rise in hemoglobin, namely, from 13 to 40 per cent.

CASE 7.—The following history was supplied by Dr. H. R. Peters of Mercy Hospital, to whom the blood samples were originally submitted for examination to explain a hemolytic transfusion reaction.

In 1937, on the day following that of a cesarean operation the patient was given a blood transfusion, the husband acting as donor. This transfusion was

followed by a chill and a rise in temperature to 101 F., but there was no hemoglobinuria. Sept. 6, 1940, one month after a cesarean operation had been performed at a hospital in Maryland, the patient was given another transfusion of her husband's blood. Thirty minutes after the transfusion the patient had a severe chill, and this was followed by a rise in temperature to 105 F. Hemoglobinuria was noted after five hours, and this was associated with oliguria, the total urinary output during the next day being only 130 cc. The icterus index rose to 18, and the blood nonprotein nitrogen to a peak of 89 mg. per hundred cubic centimeters. The patient was given sodium lactate, a 700 cc. plasma transfusion and daily intravenous infusions. Her condition gradually improved, and she was discharged from the hospital in apparent good health.

Tests made on blood samples sent to me one month after the transfusion gave the following information:

Blood	Group	Type	Rh Reaction
Patient's	O	M	Negative
Husband's	O	M	Positive
Infant's	O	M	Positive

No isoantibodies could be demonstrated in the patient's serum, but there seems to be no doubt that here again one is dealing with a hemolytic reaction caused by the Rh factor.

As a result of the experiences in cases 3, 6 and 7, I now use donors of Rh-negative blood (of the same group as the patient) routinely for postpartum transfusions.[18] (As already indicated in the reports of cases 4 and 5, this procedure is also followed for patients having reactions after repeated blood transfusions, unless the Rh factor can be excluded as the cause of the reactions.) When donors of Rh-negative blood are not available, donors of compatible blood are selected with the aid of the centrifuge technic of direct matching, performed at refrigerator, room and body temperatures. In view of the findings described in cases 5, 6 and 7 it is clear that even this method of matching is not entirely dependable for the selection of donors of compatible blood, since hemolytic transfusion reactions can occur despite the absence of demonstrable isoantibodies from the patient's serum.

In conclusion, 3 additional cases in which hemolytic transfusion reactions were caused by isoimmunization against agglutinogen Rh will be briefly described.

Case 8.—The blood samples in this case were referred to me for study by Dr. Charles R. Drew.

Dec. 2, 1939 a 12 year old girl was admitted to the Presbyterian Hospital with signs and symptoms of nephrosis. During January 1940 the patient, who belonged to group A, was given transfusions totaling 850 cc. of group O bank blood, taken from 3 different donors. There was no reaction or at most only slight reactions to these blood transfusions, but the hemoglobin dropped instead

18. This procedure is also followed by Levine.

of rising after the transfusions. March 16 a transfusion of 200 cc. of group A bank blood was followed by a chill, fever and a further drop in hemoglobin. March 25 a biologic test with 20 cc. of blood from a group A donor was tried, and when no reaction occurred after two hours, 150 cc. of blood from this donor was transfused. After the transfusion the patient exhibited marked flushing of the face, fear, dyspnea and then a chill. The next day she was noticeably jaundiced and the urine contained hemoglobin. May 21 a transfusion of 100 cc. of group A blood was given, and this resulted in a severe chill and a rise in temperature to 107 F. The patient died May 23.

The bloods of the patient and the last donor, tested the day following the final transfusion, showed the following:

Blood	Group	Subgroup	Type	Rh Reaction
Patient's	A	A_1	MN	Negative
Donor's	A	A_2	M	Positive

By the M,N tests it was readily proved that the reaction after the last transfusion was due to hemolysis of the donor's blood. Since the tests were made only one day after the transfusion, it is not surprising that no isoantibodies were found in the patient's serum, but the fact that the patient's blood was Rh negative and the donor's blood Rh positive supports the impression that the hemolysis is related to the Rh factor.

Incidentally, this case demonstrates that the biologic test is not always reliable when carried out according to the technic usually recommended. As has been pointed out by Wiener and Schaefer,[3] the hemolysis even of huge quantities of transfused incompatible blood may be entirely symptomless. A more dependable biologic test is the demonstration of the survial of the donor's blood in the patient's circulation after the transfusion. Where group O donors are used for patients of groups A, B and AB, this is readily done by demonstrating the presence of partial agglutination in the usual grouping tests on the patient's blood after the transfusion. When blood of the homologous group is transfused, the M,N reactions can be used for this purpose, as has been described in the cases reported in this article and in those reported by Wiener and Peters.[1]

Case 9.—The clinical history and blood samples in this case were sent to me by Dr. Thomas H. Ham, of Boston.

The patient, who belongs to group A, had her first transfusion from her husband, who belongs to group O, in 1937, without any untoward reaction. Her second transfusion, in October 1940, also from her husband, resulted in only a moderately severe reaction. A third transfusion, 500 cc. from another donor, also of group O, Jan. 4, 1941, was symptomless. The fourth transfusion, January 7, of 200 cc., again from her husband, resulted in an immediate febrile reaction, hemoglobinemia, hemoglobinuria and increasing anemia. The next day, January 8, a transfusion of 300 cc. from a group A donor was followed by a rise in temperature to 105 F. and hemoglobinuria.

A search for isoagglutinins was carried out by Dr. Ham, and positive reactions were obtained by the centrifuge technic at refrigerator temperature. Tests made by me on samples of the patient's blood received by mail were negative, prob-

ably because of the rapid deterioration of the weak isoagglutinin. Grouping tests on the bloods of the patients and the three donors revealed the following:

Blood	Group	Subgroup	Type	Rh Reaction
Patient's	A	A_1	M	Negative
Husband's (donor for 1st, 2d and 4th transfusions)	O	..	MN	Positive
Second donor's (for 3d transfusion	O	..	MN	Negative
Third donor's (for 5th transfusion)	A	A_1	M	Positive

The fact that the blood of the patient and that of the donor selected for the third transfusion were both Rh negative, while the bloods of the husband and the donor for the fifth transfusion were Rh positive, corresponds with the clinical observations that aside from the initial sensitizing transfusion in 1937, the third transfusion was the only one not followed by a reaction.

Case 10.—The patient, a man 34 years of age, is still under observation at the Jewish Hospital of Brooklyn.

He was admitted to the hospital in 1933 for ulcerative colitis. While in the hospital he received a transfusion of 500 cc. of compatible group O blood, and this transfusion, as well as two or three other transfusions he received at home, was not followed by any untoward reaction. Jan. 10, 1941 a resection of the colon was carried out, and postoperatively the patient was given 500 cc. of group O blood. January 15 a transfusion of 500 cc. of group O blood was followed by a rise in temperature from 102 F. to 105 F. and icterus, the latter being attributed by the clinicians to postoperative peritonitis. January 19 another transfusion of 500 cc. of fresh, citrated blood from a different donor was followed by a chill, a rise in temperature to 105 F., hemoglobinemia, hemoglobinuria and increasing icterus. The nature of the reaction was then recognized, and no further transfusions were given. There was no suppression of urination, and the patient recovered from the transfusion reaction without any sequelae aside from a drop in hemoglobin to 65 per cent from the preoperative level of 85 per cent.

Examination of the blood of the patient and of that of the donor whose blood was used for the last transfusion produced the following facts:

Blood	Group	Type	Rh Reaction
Patient's	O	MN	Negative
Donor's	O	M	Positive

With the M,N test it could be shown that about 80 per cent of the blood received from the donor was hemolyzed immediately after the transfusion and that the balance was eliminated within twenty-four hours. The tests for isoantibodies in the patient's serum were negative even by the centrifuge technic, although several blood samples were tested during the week following the last transfusion. However, an interesting phenomenon was observed when small test tubes were set up containing mixtures of 2 drops of patient's serum and 1 drop of blood suspension and allowed to stand for three hours at room temperature. In tests on Rh-negative blood the sediment was found to be compact and had a sharp, perfectly circular outline; on the other hand, Rh-positive blood gave a more diffuse sediment with an irregular outline, so that the sediment had a wrinkled appearance. Dr. Karl Landsteiner informs me that he has also observed this phenomenon in tests on certain post-transfusion serums.

COMMENT

The observations described in this paper supply an explanation for the conflicting clinical results obtained by different clinicians in the treatment of blood dyscrasias or other diseases when repeated blood transfusions are used.[19] The unsatisfactory results obtained by some investigators [20] can probably be attributed to inapparent hemolysis of the donor's blood or to a frank hemolytic reaction, due to the presence in the patient's serum of isoantibodies giving reactions independent of the blood groups. The experiences described in this paper indicate that the majority of such isoantibodies correspond to the property Rh. The use of donors of Rh-negative blood in such instances will prevent severe and possibly fatal reactions and, furthermore, will be found more effective in correcting the anemia, as in case 5 described in this paper. The occurrence in postpartum patients of occasional severe hemolytic reactions when transfusions of the proper group are given is explained in a similar way. Most of these accidents appear to be due to the presence of Rh isoantibodies in the mother, the fetus in utero being the source of the antigen which stimulates the production of these immune bodies. Accordingly, when possible, donors of Rh-negative blood should be selected for the transfusion of blood to such patients.

While, as a rule, I use only donors of the same blood group as the patient for transfusion, in a few of the cases described in this paper donors of group O were used for patients of other groups. The use of group O persons as "universal donors" was first suggested by Ottenberg,[21] who showed that while transfusions of blood containing incompatible agglutinogens in the blood cells usually resulted in a hemolytic reaction, transfusions of blood containing incompatible agglutinins in the plasma were as a rule uneventful, probably because the foreign agglutinins were immediately diluted on entering the patient's blood stream. Levine and Mabee [22] warned that group O persons with exceptionally high titers of agglutinins (or hemolysins) should be avoided as "dangerous universal donors," since the dilution of their potent isoantibodies might not be sufficient to prevent hemolysis of the patient's cells. Certain reports of hemolytic transfusion reactions following the indiscriminate use of universal donors support their contention. However, the cases described in this paper demonstrate that hemolytic reactions following transfusions from universal donors to patients not belonging to group O are not always explainable in this way, since in

19. Wiener, A. S.: Blood Groups and Blood Transfusion, ed. 2, Springfield, Ill., Charles C. Thomas, Publisher, 1939.

20. Sharpe, J. C., and Davis, H. H.: J. A. M. A. **110**:2053, 1938.

21. Ottenberg, R.: J. Exper. Med. **13**:425, 1911.

22. Levine, P., and Mabee, J.: J. Immunol. **8**:425, 1923.

our cases the hemolysis involved the donor's blood cells, instead of the patient's cells, owing to the presence in the patient's serum of Rh isoantibodies acting on the donor's cells which contained the Rh agglutinogen. Hemolysis of the blood cells from universal donors may also occur in a patient of subgroup A_1B (or A_1) in the rare instances when the recipient's serum contains natural or immunizational anti-O isoantibodies.[23]

That a hemolytic transfusion reaction following the use of a universal donor for a patient not belonging to group O is due to the isoantibodies in the donor's serum acting on the patient's cells, as suggested by Levine and Mabee,[22] can be established by showing that it is the patient's cells and not the donor's that are hemolyzed. For example, if the patient belongs to group A, his blood should consist of a mixture of group A and group O blood after the transfusion, despite the hemolytic reaction. In the cases described in this paper (cases 9 and 10) in which hemolytic reactions followed transfusions from universal donors to patients not belonging to group O, it was the donors' blood that was hemolyzed. In fact, for some patients (e. g., those in cases 4 and 5) transfusions from universal donors selected because their blood is Rh negative are safer than transfusions from donors who are of the same group as the patient but otherwise selected at random. In the light of these newer findings, therefore, a person of group O with Rh-negative blood and weak isoagglutinins in his serum would be a "safe universal donor" except possibly for those very rare recipients of subgroups A_1 and A_1B whose serums contain anti-O isoantibodies.

SUMMARY

Ten cases have been described in which hemolytic reactions followed transfusions of blood of the same group as that of the patient (in 2 of these cases reactions also occurred following transfusions from "universal donors"). In 4 cases irregular isoantibodies were detected in the patients' serums. Three of these serums behaved alike, giving agglutination reactions corresponding to the property Rh of Landsteiner and Wiener. Moreover, the patients in these cases all had Rh-negative blood and the incompatible donors Rh-positive blood, and the patients' serums clumped the cells of the donors of the incompatible blood. Accordingly, these cases belong in the same category as those previously reported by Wiener and Peters and by Levine; i. e., they are instances in which hemolytic transfusion reactions were caused by individual differences with respect to property Rh. In the fourth case with demonstrable isoantibodies the serum reactions were different from those of the standard Rh serums, although there was a correlation between them.

23. Wiener, A. S.; Hyman, M. A.; Oremland, B. H., and Samwick, A. A.: Am. J. Clin. Path. **11**:102, 1941.

The reactions given by this serum indicate the existence of more than one sort of agglutinogen Rh, in analogy, for example, to the varieties of agglutinogen A. A review of all the cases of intragroup isoimmunization with demonstrable isoantibodies which have been encountered by me to date (including those previously reported with Peters) revealed that in 7 the Rh agglutinogen was involved, in 2 the agglutinogen A_1 and in 1 the agglutinogen P.

The 6 cases reported in this paper in which hemolytic transfusion reactions occurred but in which no isoantibodies were demonstrable in the patients' serum, even by the centrifuge technic at refrigerator, room and body temperature, offered a special challenge.[24] In view of the findings in cases in which isoantibodies were demonstrable, it seemed plausible that in most of these 6 cases also, the hemolytic reactions would prove to be due to isoimmunization to factor Rh. That this surmise was correct was indicated by the fact that the blood of all 6 patients was Rh negative, while only 1 of every 7 persons taken at random has blood free from this factor. Moreover, in 2 patients deliberate transfusion of Rh-negative blood was followed by a satisfactory clinical response each time, while transfusions from unselected donors (presumably with Rh-positive blood) of the same group as the patient resulted in hemolytic transfusion reactions.

The 10 cases described in this paper (and as Wiener and Peters[1] point out, the same is true of the cases reported in the literature) fall into two categories. In one group of cases the patients were given repeated blood transfusions, the earlier transfusions serving to immunize them against the Rh antigen, and the later transfusions giving rise to the hemolytic reactions. In the other group of cases the patients had hemolytic reactions following their very first transfusions. In every instance of the latter group the reaction occurred in an intrapartum or postpartum patient, the source of the antigen that immunized the patient having been the fetus in utero.[6] It is advocated, therefore, that in such instances, whenever possible, Rh-negative blood (of the same group as that of the patient) should be used for transfusion. It is pointed out that in general a "safe universal donor" would be a person of group O with Rh-negative blood and only low-titered isoagglutinins in his serum.

The observations described in this paper supply an explanation for the erratic clinical results sometimes observed when treating diseases (particularly blood dyscrasias) in cases in which repeated blood transfusions are required. It is pointed out that in the careful selection of compatible blood donors in such instances special attention to the Rh factor may result in more satisfactory clinical results from the transfusions.

24. Similar cases have been observed by Dr. P. Levine (personal communication).

ANOMALIES OF THE CIRCLE OF WILLIS IN RELATION TO CEREBRAL SOFTENING

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Anomalies of the posterior communicating branches of the arterial circle of Willis and their clinical significance have received scant attention in medical literature. The few references available are, with occasional exceptions, concerned with purely developmental or statistical studies. Some of them deal with the relationship of such anomalies to mental disease or to criminality or to both.

The practical import of anomalies of the circle of Willis has been stressed by Saphir,[1] who in 1935 reviewed the literature and suggested that defects in the circle of Willis are responsible for cerebral softening and hemorrhage in certain cases. He referred particularly to anomalies of the posterior communicating branches. Saphir explained that cerebral softening and hemorrhage in the absence of definite occlusive thrombotic lesions of the cerebral arteries could sometimes be accounted for by the coexistence of congenital circle defect, heart failure and cerebral arteriosclerosis. He reported autopsies in 2 cases in each of which this triad was present in conjunction with cerebral softening.

Saphir criticized the "increasing tendency today to attribute to functional abnormalities the anatomical changes that are undoubtedly the result of vascular lesions," referring particularly to theories of Fischer-Wasels and of Weil, in regard to the genesis of cerebral softening and hemorrhage.

ROUTINE AUTOPSY EXAMINATION OF THE CIRCLE OF WILLIS

Because of Saphir's interesting explanation of the cerebral softening in his 2 cases, we began, several years ago, to examine brains encountered in the autopsy service at the Pittsburgh City Home and Hospitals, with special attention to the circle of Willis. We were surprised to find poor

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1. Saphir, O.: Am. J. Path. **11**:775, 1935.

collateral circulation in many instances. We soon found several cases similar to those described by Saphir, i. e., showing cerebral softening in association with cerebral arteriosclerosis, congenital defect of the circle of Willis and heart failure, without demonstrable occluding arterial lesions.

It seemed to us that the part played by a defective circle in the genesis of cerebral softening could be evaluated better by study of a series of cases in which autopsies were made and by interpretation of the statistics made available thereby than by picking out the occasional cases in which the aforementioned combination of findings were found.

The autopsy material at the Pittsburgh City Home and Hospitals is peculiarly suited for such a study because of the high average age (59 years) with the accompanying high percentage of cerebral softening.

Brains from 200 consecutive subjects of complete autopsies from February 1936 to October 1939 were carefully examined, especially from the point of view of cerebral softening and defects of the circle of Willis. One hundred and thirty-eight of the subjects were mental hospital patients. Sixty-two were general hospital patients. Multiple coronal sections 1 cm. apart were made, and foci of softening were noted and measured. Only those anomalies causing some degree of interruption of the arterial circle were considered in this study. As a matter of fact, every interruptive anomaly found involved one or both of the posterior communicating branches. This was not unexpected, as for embryologic reasons these branches are most likely to fail to develop. We included in our series of anomalies those in which we found one or both posterior communicating branches absent or so narrow as to measure definitely less than 0.5 mm. in outside diameter and to be almost or entirely impervious. Such a vessel is termed threadlike in this report.

The findings were then assembled and grouped in order to ascertain the association, if any, of defects of the circle of Willis with cerebral softening.

TABULATION AND EXPLANATION OF FINDINGS

In figure 1 may be seen schematic representations of so-called *primitive, transitional, mixed* and *normal* arterial circles. The diagrams are adapted from Saphir.[1]

The primitive circle, as illustrated, presents large posterior communicating branches, which contribute nearly all the blood supply to the posterior cerebral arteries. This type of circle has been termed primitive because in lower animals the internal carotid arteries furnish all or nearly all of the cerebral blood supply.

The transitional type, as shown here, might, in the light of our observations, be considered an unusually good circle, with capacity for interchange of blood far above the average.

The mixed circle, as illustrated, is one in which the right posterior cerebral artery is formed chiefly by the right internal carotid artery and the left posterior cerebral artery is formed principally by the left divisional branches of the basilar artery. This type of circle was frequently encountered in our series. It represents a fairly common asymmetric development, usually not resulting in interruption of the circle. Saphir [1] discussed fully the phylogenetic and ontogenetic relationships of arterial circles and the references thereto.

The normal circle, as shown, might better be termed an average circle. Considerable leeway must be allowed in judgment of the normal size of posterior communicating branches. Examples of the "normal" circle as displayed diagrammatically in the standard anatomy texts were encountered in less than 50 per cent of our 200 brains.

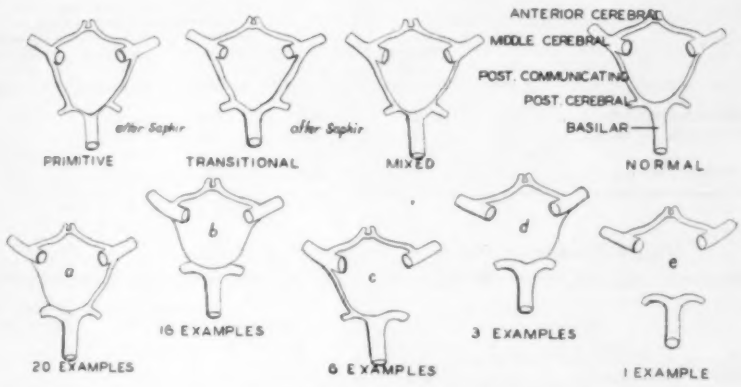

Fig. 1.—Diagrams of the types of circle of Willis and types of interruptive defect of the circle encountered in this series.

Occurrence of Interruptive Anomalies.—Of the 200 brains in our series, 46, or 23 per cent, presented anomalies of the posterior communicating branches tending to cause interruption of the arterial circle (table 1). Included in the 46 anomalies are five types of circle defect (fig. 1). Commonest is a threadlike formation of one or the other posterior communicating branch (fig. 1 *a*)—20 examples. Next in frequency is the simultaneous occurrence of threadlike posterior communicating branches (fig. 1 *b*)—16 examples. Absence of either posterior communicating branch with presence of a normal one was encountered six times (fig. 1 *c*). Absence of either posterior communicating branch with presence of a threadlike one was noted three times (fig. 1 *d*). Finally, there was complete absence of both posterior communicating branches in 1 case (fig. 1 *e*).

Sex Incidence.—Table 2 shows the distribution of the circle anomalies in reference to sex. Of 159 males, 32, or 20.1 per cent, presented anomalies, as contrasted with 14 anomalies among the 41 females, an incidence of 34.1 per cent.

Occurrence of Cerebral Softening in Association with Anomalies.—Table 1 compares figures regarding the coincidence of cerebral softening with circle anomalies. Of the entire group of 200 brains, 60, or 30 per cent, showed some degree of softening. These may be separated as to the co-occurrence of softening and anomaly; of 154 brains without circle anomalies, 44 showed softening (28.5 per cent), and of 46 brains that

TABLE 1.—*Occurrence of Softening in Relation to Anomalies of the Circle of Willis*

Group	Cases	Number with Softening	Percentage with Softening	Cases with Demonstrable Occlusive Lesions Deleted: Cases	Cases with Demonstrable Occlusive Lesions Deleted: Number with Softening	Cases with Demonstrable Occlusive Lesions Deleted: Percentage with Softening
Without anomalies.....	154	44	28.5	143	33	23.0
With anomalies.....	46	16	34.7	44	14	31.8
Total.....	200	60	30.0	187	47	25.1

TABLE 2.—*Sex Incidence of Anomalies of the Circle of Willis*

Group	Cases	Number with Anomalies	Percentage with Anomalies
Male.....	159	32	20.1
Female.....	41	14	34.1
Total.....	200	47	23.5

presented anomalies, 16 also showed softening (34.7 per cent). If we consider only those brains in which softening cannot be explained by demonstrable occlusive or traumatic lesions, these percentages become 23 and 31.8, respectively.

Occurrence of Cerebral Softening in Association with Heart Failure.—In 23 of the 60 cases of softening in this series, heart failure was present—in 18 of the 44 cases of softening without circle defect and in 5 of the 16 cases of softening with circle defect.

Thus, 5 cases in the series were of the type reported by Saphir. Two of these are illustrated in figures 2 and 3.

Figure 2 shows temporoparietal, superior frontal and internal capsular softening in a case in which cardiac decompensation was present on an arteriosclerotic basis, with a sclerotic circle and a threadlike condition of both posterior communicating branches.

Figure 3 illustrates softening of the right thalamic region, of the left midfrontal region and of the left temporal tip in a case of cerebral arteriosclerosis, heart failure and absence of the left posterior communicating branch.

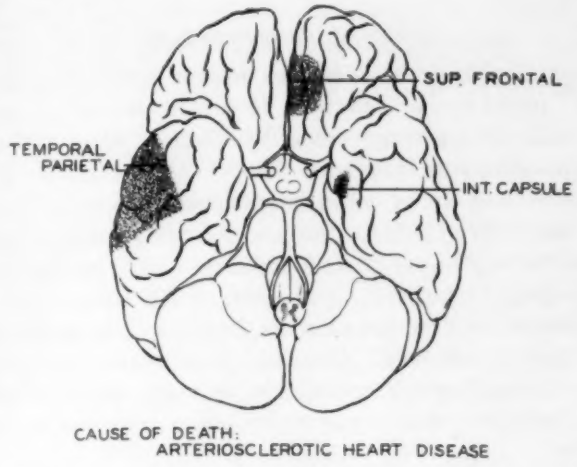

Fig. 2.—Diagram of a brain with softening and an anomaly of the circle of Willis in the presence of heart failure.

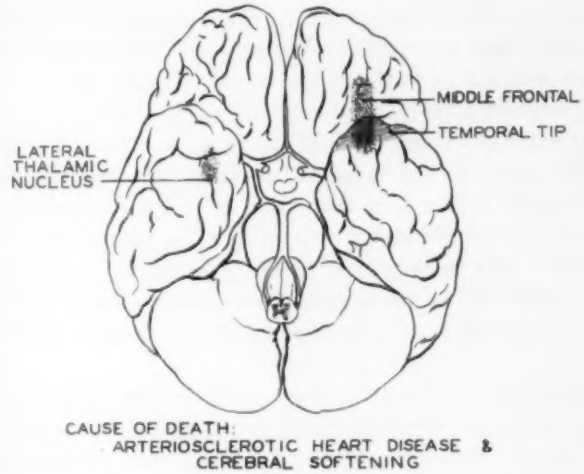

Fig. 3.—Diagram of a brain with softening and an anomaly of the circle of Willis in the presence of heart failure.

Both of these cases illustrate the triad of factors which Saphir has suggested as an occasional cause for softening.

Occurrence of Cerebral Softening in Relation to Age of Subjects and Circle Defects.—The average age of the 44 patients presenting cerebral

softening without circle defects was 65.5 years. The youngest was aged 37 years; the oldest, 84.

The average age of the 16 patients presenting cerebral softening with circle defects was 64.1 years. The individual ages ranged from 47 to 82 years.

GENERAL CONSIDERATIONS

The total incidence of anomalies in our series was 23 per cent. Blackburn [2] found similar defects in 15 of 220 consecutive patients with mental disease (6.8 per cent) and Windle,[3] in 32 of 200 consecutive patients who were free from such disease (16 per cent). In 25 of his cases Windle found one or both posterior communicating branches missing. The sex difference in the incidence of circle anomalies as shown in our series (males, 20.1 per cent; females, 34.1 per cent) is in agreement with the findings of Fawcett [4] in a series of 700 cases.

It is difficult to draw conclusions from such anatomic findings as have been herein presented. Certainly, it is interesting that the circle of Willis deviates from the normal in so many cases. Further, it is interesting that this deviation may so frequently represent an interruption of the circle.

Although Saphir was apparently the first to call attention to the possible cause and effect relation of circle defects to softening, Windle [3] had previously noted the increased severity of thrombosis in the presence of circle defects. Our own figures in regard to the association of softening and circle defect are worthy of comment. We believe it reasonable, in trying to sort out or evaluate the factors bearing on the relationship or co-occurrence of cerebral softening and circle defect, to eliminate from our observered series those cases of softening in which definite occlusive lesions were found in the vessels supplying the infarcted areas. The percentages which follow such correction of the figures are 23 for softening of brains without circle defects and 31.8 for softening of brains with circle defects. We believe that a larger series than the present one should be studied before too definite conclusions are drawn, and to that end we are examining an additional series of brains from this point of view.

It is not surprising that there seems to be a slightly greater tendency to softening in brains with interrupted circles than in those with normally protective circles. It would indeed be unlikely that the presence of such a defect would not predispose to softening.

The incidence of softening in the general group seems high. We included in the series any softening that was grossly visible. It is under-

2. Blackburn, I. W.: J. Comp. Neurol. & Psychol. **17**:493, 1907.
3. Windle, cited by Blackburn.[2]
4. Fawcett, E., and Blackford, J. V.: J. Anat. & Physiol. **40**:63, 1905.

stood, of course, that tumors or traumatic softenings were not considered. Duration of softening was disregarded, a few cysts of softening being included in this series.

SUMMARY

The value of the circle of Willis as a factor in collateral circulation is impaired in a surprisingly large percentage of cases. Arterial circles of the type generally considered normal were found in less than 50 per cent of our subjects.

Twenty-three per cent of the brains from a series of 200 consecutive subjects of autopsies showed interruptive circle anomalies involving the posterior communicating branches alone.

Cerebral softening occurred in 30 per cent of the total number of brains. Excluding those in which softening was due to demonstrable occlusive lesions and trauma, there was a definitely higher incidence of softening among the group showing anomalies of the circle of Willis than among those without such anomalies. These findings lend statistical support to Saphir's hypothesis concerning the relation of anomalies of this circle to cerebral softening.

HISTOLOGIC DEMONSTRATION OF VITAMIN A IN TUMORS

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AND
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The purposes of this investigation were primarily to ascertain the distribution of vitamin A in tumors by means of fluorescence microscopy [1] and to study the possible role of this vitamin in tumor formation. In addition, the green fading vitamin A fluorescence proved to be a morphologic characteristic of certain tumor structures.

Regarding the relationship of vitamin A and tumor formation, two schools of thought exist. In experimental vitamin A deficiency the differentiated growth of epithelium is impaired, resulting in a nonspecific proliferation, characterized by keratinization.[2] The proliferated nonspecific growth may change into tumor formation.[3] According to Burrows,[4] tumor formation takes place in the local absence of vitamin A. High doses of vitamin A supposedly [5] inhibit tumor formation.

Huzita,[6] on the other hand, believes that vitamin A is necessary for tumor growth, that a deficiency decreases and an excess increases tumor growth. Howe, Elliott and Shear [7] varied the vitamin A content of the diet and noticed no significant difference in the tumor-producing effect of 3,4-benzpyrene implanted subcutaneously in mice.

Only a few comments in the literature are made regarding the vitamin A contents of tumors as determined by chemical assay.[8] This method

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1. (*a*) von Querner, F.: Klin. Wchnschr. **14**:1213, 1935. (*b*) Popper, H.: J. Mt. Sinai Hosp. **7**:119, 1940. (*c*) Popper, H., and Greenberg, R.: Arch. Path. **32**:11, 1941.

2. Wolbach, S. B.: J. A. M. A. **108**:7, 1937. Schour, I.; Smith, M. C., and Hoffman, M. M.: Proc. Soc. Exper. Biol. & Med. **39**:447, 1938.

3. Orten, A. U.; Burn, C. G., and Smith, A. H.: Proc. Soc. Exper. Biol. & Med. **36**:82, 1937.

4. Burrows, M. T.: J. Cancer Research **10**:239, 1926.

5. Kuh, C.: Yale J. Biol. & Med. **5**:123, 1932. Dittmar, C.: Ztschr. f. Krebsforsch. **44**:73, 1936. Lustig, B., and Wachtel, H.: ibid. **44**:53, 1936.

6. Huzita, Y.: Jap. J. Obst. & Gynec. **21**:370, 1938; **22**:38, 1939.

7. Howe, P. R.; Elliott, M. D., and Shear, M. J.: Am. J. Path. **16**:295, 1940.

8. Vogt, E.: Med. Klin. **28**:1344, 1932. Huzita.[6]

is of minor importance, since the vitamin A found chemically in a tumor may not be distributed in the tumor cells per se but in non-specific structures, such as fat cells.

In the normal tissues vitamin A has been localized by fluorescence microscopy in certain structures, e. g., the Kupffer and epithelial cells of the liver, the fascicular and glomerular layer of the adrenal cortex, the gonads, fat tissue and the lactating breast.[1a]

MATERIAL AND METHODS

In all, 219 tumors were studied. The first 150 specimens were taken from the routine material submitted for surgical pathologic examination and the remaining 69 from selected biopsy and postmortem material.

TABLE 1.—*Distribution and Vitamin A Fluorescence of the Tumors Studied*

	Malignant Tumors		Benign Tumors		Granulomas		
Organ or System of Organs	Vitamin A Positive	Fluorescence Negative	Vitamin A Positive	Fluorescence Negative	Vitamin A Positive	Fluorescence Negative	Total
Adrenal gland	..	1	6	..	..	..	7
Breast	7	9	1	8	..	..	25
Bone and tendon sheath	1	4	2	1	..	..	8
Female sex organs	3	8	13	31	..	..	55
Gastrointestinal tract	6	16	..	..	..	..	22
Kidney-urinary bladder tract	18	2	..	3	..	..	23
Larynx and pharynx	1	4	..	4	..	..	9
Liver and gallbladder	6	14	..	1	..	..	21
Lung	..	2	..	..	..	..	2
Lymph nodes	..	8	..	..	..	5	13
Parathyroid gland	..	..	..	1	..	..	1
Prostate	1	1	..	6	..	..	8
Retroperitoneal region	2	1	..	..	..	..	3
Skin	2	6	2	4	..	..	14
Testicle	4	..	..	..	..	..	4
Thyroid gland	..	1	1	2	..	..	4
Total	51	77	25	61	0	5	219

Tissues were fixed in solution of formaldehyde U. S. P. (diluted 1:10) for a period not exceeding twenty-four hours, and frozen sections were cut 10 to 20 microns thick. Examinations for spontaneous fluorescence of vitamin A by means of the fluorescence microscope were made. In addition, the fat content of the tumors was studied by fluorescing stains (phosphin 3 R). Methylene blue stained-sections were studied in ultraviolet rays to visualize the vitamin A fluorescence and in visible light to localize the effect. The tumors were examined for anisotropic lipoids with Nicol prisms. Details of the technic were presented in a previous paper.[1b]

Routine studies in visible light were also made.

FINDINGS AND ANALYSIS

The majority of tumors studied did not reveal vitamin A fluorescence in the tumor parenchyma (table 1). If vitamin A was found only in fat droplets of the stroma or fat cells, the tumor was considered devoid of vitamin A. In many of the cases in which vitamin A fluorescence was not found, fat was demonstrated either in the parenchyma or

in areas of fatty degeneration. In most of the tumors, irregularly shaped bodies of yellow, brown or red fluorescence were encountered. The study of these various fluorescent details may be of interest, but their significance has not been established and consequently they have not been included in this study.

Of 219 tumors, 76 showed vitamin A fluorescence (table 2). The positive findings belonged to a certain group of tumors. Most of these

TABLE 2.—*Estimation of the Total Amount of Vitamin A Fluorescence in the Positive Tumors*

Organ	Tumor Type	Tumors	Amount
Adrenal gland...	Benign adenoma	6	+++ to ++++
Bone	Metastasis of a hypernephroma	1	+ to ++
Breast	Adenocarcinoma	2	+ to ++
	Carcinoma simplex	3	+
	Intraductal carcinoma	2	+ to ++
	Tubular adenoma	1	++++
Gastrointestinal tract	Adenocarcinoma of cecum	3	+ to ++
	Adenocarcinoma of the descending colon	1	+ to ++
	Adenocarcinoma of the stomach	1	± to +
	Malignant carcinoid of the ileum	1	± to +
Kidney	Hypernephroma	18	+ to ++++
Liver	Hepatocellular carcinoma	5	± to +++
	Metastasis of a hypernephroma	1	+ to ++
Larynx	Squamous cell carcinoma	1	+
Ovary	Arrhenoblastoma	1	++ to +++
	Dysgerminoma	1	++ to +++
	Granulosa cell tumor, benign	4	++ to ++++
	Granulosa cell tumor, malignant	1	++ to ++++
	Malignant teratoma	1	+ to ++
	Theca cell tumor	1	++ to ++++
	Xanthofibroma	4	+++ to ++++
Prostate	Plant cell carcinoma	1	++++
Retroperitoneal region	Extra-adrenal tumor	1	+ to ++++
	Lipoblastoma	1	+ to ++
Skin	Lipoma	2	++ to ++++
	Lipofibrosarcoma	1	++
	Squamous cell carcinoma	1	++
Tendon sheath...	Benign giant cell tumor	2	++++
Testicle	Dysgerminoma	4	+ to +++
Thyroid glands..	Tubular adenoma	1	++++
Uterus	Adenomyosis	2	+ to ++
	Sarcoma of intramural endometrial stroma	1	+++ to ++++
Total		76	

arose from parent tissues which either usually or under certain circumstances contain vitamin A.

Liver.—Normally the liver is rich in vitamin A. Two of 5 hepatocellular tumors diagnosed as carcinoma showed moderate vitamin A fluorescence in the tumor cells, whereas the Kupffer cells in all 5 tumors contained vitamin A. In a hepatocellular tumor diagnosed as adenoma (hamartoma) no vitamin A was found. The surrounding liver tissue was rich in vitamin A. Hepatic metastases which were free of vitamin A at their primary site did not show vitamin A fluorescence except for

an occasional Kupffer cell which was still apparent in the tumor metastasis.

Adrenal.—The normal adrenal shows vitamin A fluorescence in the glomerular and especially in the fascicular layer of the cortex. The fluorescence is often encountered in combination with anisotropic lipoids. Of the 6 benign tumors diagnosed as cortical adenoma of the adrenal gland, all were extremely rich in vitamin A fluorescence (fig. 1 *A*) and in double refractile lipoids. The picture resembled the glomerular layer of the normal adrenal gland. The vitamin A fluorescence was seen in fine droplets in the cytoplasm of the cells and was invariably present, although occasionally the vitamin A content in the remaining normal adrenal was depleted or markedly reduced. Adenomatous hyperplasia of the adrenal showed a picture similar to that of the encapsulated adenoma.

Adipose Tissue.—The fat tissue normally shows vitamin A fluorescence in small amounts. The same slowly fading fluorescence was seen in lipoma in several instances in relatively smaller amounts. In 2 specimens diagnosed as fibroliposarcoma, lipoid droplets imparted a moderate vitamin A fluorescence. The cytoplasm of the tumor cells resembled that of mature fat cells.

Testicle.—Normally in the mature testicle a moderate amount of vitamin A fluorescence is revealed in fine droplets in the tubular cells. It is also found in greater amounts in the Leydig cells. Four tumors diagnosed as dysgerminoma of the testicle showed moderate vitamin A fluorescence. In the intact tumor cells it was present in fine droplets, with uniform distribution. The distribution resembled that of the tubular cells in the uninvolved portion of the testicle. These fine fluorescent droplets were distinctly differentiated from the larger droplets of the Leydig cells, which contained anisotropic lipoids. One of these tumors arose in an undescended testicle of a 12 year old boy. The Leydig cells surrounding the tumor showed vitamin A fluorescence in larger droplets localized in the cytoplasm of the cells but did not contain the usual brown fluorescent pigment that is seen in cells only during active sex life.

Ovary.—The mature normal ovary showed the vitamin A fluorescence in three anatomic sites. The phenomenon was found (1) in certain stages in the cortical stroma, in finely dispersed, quickly fading green fluorescent droplets, (2) in medium-sized droplets in the granulosa cells of the corpus luteum and (3) in the theca lutein cells, where it was seen in large homogeneous droplets. The latter were less fluorescent and more slowly fading than the quickly fading droplets in the cortex and in the luteinized granulosa cells. Strong double refraction was also seen in the theca cells.

The vitamin A distribution in the ovarian tumors varied according to the type of tumor. In 8 ovarian tumors of two common types multilocular serous and mucinous papillary cystoma, vitamin A fluorescence was seen only in the stroma composed of the original structure of the ovary. The tumor epithelium did not show vitamin A, although lipoid droplets were found in areas of regressive metamorphosis. These tumors supposedly do not arise from ovarian tissue.[9]

Vitamin A fluorescence was frequently observed in solid ovarian tumors. Among the ovariogenic tumors three groups of feminizing mesenchymatous tumors revealed vitamin A fluorescence in a distribution resembling all three types of the distribution seen in a normal ovary.

Group 1. In 6 specimens of granulosa cell tumor (one of them a malignant form) vitamin A fluorescence was present in high amounts but in an irregular distribution. Large areas were free from lipoids and vitamin A. In other areas vitamin A fluorescence was seen in numerous small to medium-sized droplets where the tumor cells showed vacuolated cytoplasm, as seen in paraffin sections (fig. 2 *C*). This picture of patchy luteinization paralleled the description of Schiller[10] and Greenhill and Greenblatt.[11]

One of the 6 tumors, a mature folliculoma type, showed an apparent similarity of the granulosa cells of the tumor to those of the corpus luteum. The vitamin A-containing droplets were localized chiefly on the periphery of the cells. In addition, irregularly shaped granules of green and brown ultraviolet stable fluorescence were seen. The lipoid droplets carrying vitamin A did not always give the phosphin 3R fat reaction as observed in the mature stages of the normal corpus luteum.

In the 5 remaining tumors not all of the fat bodies present revealed vitamin A fluorescence. It was absent in necrotic areas and some of the tumor cells, as shown by the phosphin 3R reaction. Many epithelial-like cells of granulosa cell tumors were free of lipoids and vitamin A. The fluorescence microscopic picture of the lipoid–containing areas, in unstained material, as well as after staining with phosphin 3R, resembled that of the granulosa cell layer of the corpus luteum (fig. 2 *D*). No anisotropic lipoids were seen in the granulosa tumor cells.

In addition, there were seen scattered or irregular groups of cells each of which contained a large anisotropic fat droplet imparting a

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9. Schiller, W.: Surg., Gynec. & Obst. **70**:773, 1940. Novak, E.: Gynecological and Obstetrical Pathology with Clinical and Endocrine Relations, Philadelphia, W. B. Saunders Company, 1940.

10. Schiller, W.: Pathologie und Klinik der Granulosazelltumoren, Vienna, Wilhelm Maudrich, 1934.

11. Greenhill, J. P., and Greenblatt, R. B.: Am. J. Obst. & Gynec. **36**:684, 1938.

fainter but a more slowly fading vitamin A fluorescence. After the fading, a brown fluorescent pigment appeared. These cells closely resembled the fluorescence microscopic picture of the theca cells of the corpora atretica (fig. 2 *E*).

Group 2. The presence of theca cells in granulosa cell tumors has been mentioned by various authors.[12] These cells were found in experimentally produced, biologically active ovarian tumors in mice. In the specimen in our series the double refractile theca cells were prevalent among a few granulosa-like cells, and the tumor may be classified as a true theca cell tumor.

Group 3. A third group of feminizing tumors showed a fluorescence microscopic picture entirely different from those of the former two groups, among which many transitional forms existed. The third group (3 tumors) showed in the section stained with hemalum and eosin the typical structure of fibroma of the ovary. Whereas ordinary fibroma of the ovary with the white cut surface was free of vitamin A and lipoids (as found in 2 specimens), these tumors showed large amounts of vitamin A in fine isotropic phosphin 3R–positive droplets, which were spread uniformly over the fibrous tissue. The sprinkled distribution of the vitamin A fluorescence was identical with that found in the luteinized cortical stroma. The gross cut surface showed a yellow color. These findings justify the classification of these lipoid-carrying tumors as xanthofibroma (Löffler and Priesel[13]). They apparently originated from the cortical ovarian stroma. Our tumors showed evidence of only slight endocrine activity. The fluorescence microscopic examination helped to differentiate these tumors, which were classified as xanthofibroma, from the theca cell tumors. The name "xanthofibroma thecocellulare" is not justified, for these tumors originate from the fibrous stroma of the ovarian cortex.

A fourth tumor did not completely fit the typical picture of xanthofibroma, although macroscopically a poorly defined cherry-sized yellow nodule was seen within the ovary. Microscopically, no sharp demarcation of the tumor from the ovarian stroma was evident, and a sprinkled distribution of vitamin A was seen. Within the nodule there was a typical atretic follicle. This finding suggested a transition between hyperplasia of the cortical stroma and xanthofibroma. Löffler and Priesel mentioned two types of tumors in the group which they considered as fibroma thecocellulare xanthomatodes; one type, judging from our findings, was a theca cell tumor and the other a true xanthofibroma.

12. Traut, H. F.; Kuder, A., and Cadden, J. F.: Am. J. Obst. & Gynec. **38**: 798, 1939. Geist, G. H.; Gaines, J. A., and Pollack, A. D.: ibid. **38**:786, 1939. Geist, G. H., and Gaines, J. A.: ibid. **35**:39, 1938.

13. Löffler, E., and Priesel, A.: Beitr. z. path. Anat. u. z. allg. Path. **90**:199, 1933; Wien. med. Wchnschr. **84**:400, 1934.

Of the masculinizing tumors, only one specimen, classified as arrhenoblastoma, was examined. It revealed immature trabecular and adenomatous structures. The vitamin A fluorescence was found in large cells only. These contained medium-sized and large droplets which showed strong double refraction and a positive phosphin 3R reaction. After fading of the vitamin A fluorescence, a brownish fluorescence becomes visible. The fluorescence microscopic picture suggested that these cells, which were present in small aggregations, were analogous to the Leydig cells. In addition to the aforementioned findings, isotropic lipoid droplets without vitamin A fluorescence and partly filled with brown fluorescent substances were seen in the tubular epithelium, in the lumen and in the stroma. In the adenomatous areas a resemblance to the fluorescence microscopic picture of Sertoli cells was seen.

One tumor, classified as dysgerminoma of the ovary (originating from undifferentiated mesenchymatous tissue and without endocrine function [14]), showed vitamin A fluorescence in fine droplets in some of the specific tumor cells arranged in nests and cords. The droplets were stained with phosphin 3R and did not reveal double refraction. In addition brown fluorescent pigment was visible.

In a tumor diagnosed as malignant teratoma of the ovary small amounts of vitamin A fluorescence were found in the squamous epithelial lining of a cyst (fig. 2 *B*).

In a tumor diagnosed as fibroepithelioma (Brenner's tumor) neither vitamin A nor fat was found in the epithelium or the fibrous stroma.

Uterus.—Tumors classified as fibromyoma of the uterus, even those with fatty degeneration, as demonstrated by sudan 3 or phosphin 3R, did not reveal vitamin A. In 2 specimens of adenomyosis uteri, moderate amounts of vitamin A fluorescence were seen in small lipoid droplets in the stroma, while others were free of vitamin A. A tumor diagnosed as sarcoma uteri, probably originating from an intramural endometrial stroma, a rare type of tumor, revealed large amounts of fine lipoid droplets with vitamin A fluorescence. Studies of endometrium in different stages of the menstrual cycle showed sometimes the presence of vitamin A in the stroma. These studies will be published at a later date.[15]

Breast.—An example of vitamin A fluorescence occurring in tumors which originate from parent tissues containing vitamin A under certain conditions was found in the breast. Seven tumors classified as pericanalicular and intracanalicular fibroadenoma of the breast, one of them occurring in the male breast, did not show vitamin A. One tumor diagnosed as tubular adenoma of the breast revealed large amounts of

14. Meyer, R.: Am. J. Obst. & Gynec. **28**:302, 1934.

15. Ragins, A. B., and Popper, H.: To be published.

fine fat droplets and showed vitamin A fluorescence of the cytoplasm of the epithelial cells. The picture was similar to that of the lactating breast.

Vitamin A fluorescence was demonstrated in 7 of 16 tumors grouped as carcinoma of the breast, namely, 2 specimens of adenocarcinoma, 2 of intraductal carcinoma and 3 of carcinoma simplex. In these 7 tumors vitamin A fluorescence was seen in large plantlike cells. The vitamin A fluorescence of these cells was very low, and the cells were scattered irregularly throughout the tumor. In contrast to the tubular adenoma, described in the foregoing paragraph, there was no similarity between the distribution of vitamin A in the malignant tumor and that in the normal lactating breast.

Miscellaneous.—In 8 tumors diagnosed as carcinoma, other than the malignant mammary tumors, small amounts of vitamin A fluorescence were localized in plant cells, which were easily recognized under visible light. These cells were isolated or scattered in small nests. They were large, and each contained a relatively small nucleus in the center with ample vacuolated cytoplasm, rich in fat droplets. In some of these droplets vitamin A fluorescence was demonstrated. We found this picture in a tumor of the stomach (adenocarcinoma), in a tumor of the ascending colon (adenocarcinoma), in 3 tumors of the cecum (adenocarcinoma), in a tumor of the skin (anaplastic squamous cell carcinoma) and in a tumor of the prostate gland (plant cell carcinoma), which grossly had a yellow color. Except for the malignant tumor of the prostate, in the cells of which vitamin A fluorescence was abundant, the vitamin A fluorescence was low.

Three other tumors in our series revealed vitamin A fluorescence: (1) a malignant carcinoid tumor of the ileum; (2) 2 benign tendon sheath tumors, in which the fluorescence was limited to the xanthoma cells; (3) a microfollicular adenoma of the thyroid gland with some tubular and trabecular areas. In the last tumor all epithelial cells contained large numbers of lipoid droplets rich in vitamin A fluorescence.

Kidney.—Of so-called hypernephroid carcinoma, 20 specimens were studied. In the specimens obtained at postmortem examination the metastatic as well as the primary growths were investigated. One extrarenal tumor of hypernephroid structure, localized between the kidney and the adrenal, and a biopsy specimen of a metastasis of a hypernephroid carcinoma to bone were included in this series. In all these specimens some of the tumor structures revealed varying amounts of vitamin A fluorescence, and in all but 3, large amounts of vitamin A fluorescence. The fluorescence was found in a varying distribution:

(*a*) The plant cells (fig. 1 *D*), which were considered characteristic for hypernephroid carcinoma, contained isotropic and usually aniso-

tropic lipoids. In all specimens in which these cells were found, some revealed vitamin A fluorescence; occasionally, large areas composed of these cells were free of the fluorescence. In visible light no difference could be seen between the vitamin A–containing and the vitamin A–free plant cells.

(*b*) Another characteristic arrangement of the tumor cells was in cords, which in visible and particularly in the fluorescent light showed

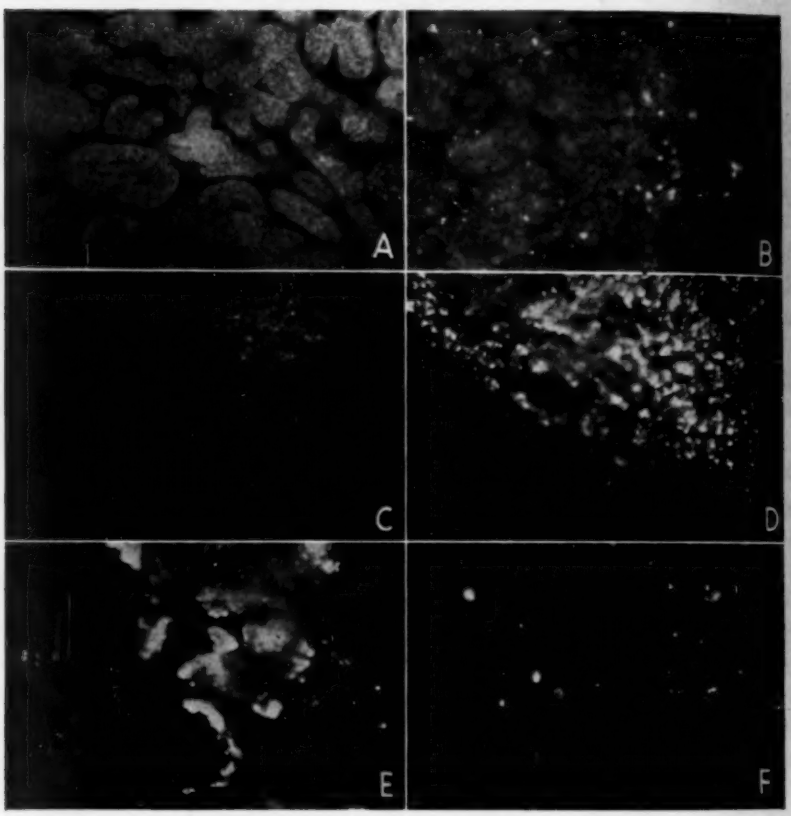

Fig. 1.—Fluorescence photomicrographs. *A*, benign cortical adenoma of the adrenal, showing vitamin A fluorescence in the epithelial cells of the trabeculated tubular structures. *B*, hypernephroma with vitamin A fluorescence of trabeculated cords similar to those of the cortical adenoma of the adrenal gland. *C*, hypernephroma under low power magnification, showing vitamin A fluorescence of adrenal-like structures. *D*, hypernephroma showing vitamin A fluorescence in plant cells. *E*, hypernephroma with vitamin A fluorescence in papillary structures. *F*, hypernephroma with vitamin A fluorescence in scattered groups of cells.

a resemblance to the fascicular and glomerular layer of the adrenal gland (fig. 1 *B* and *C*). These cells were stuffed with small lipoid droplets, which gave the vitamin A fluorescence and usually revealed

double refractile bodies (8 specimens). In addition, smaller groups of cells, which also resembled adrenal cortex, were seen under the fluorescence microscope. They were comparable to fragments of the fascicular layer of the adrenal (fig. 1 *F*). These cells were rich in anisotropic lipoids, which showed vitamin A fluorescence and were stained by phosphin 3R.

(*c*) Glandlike formations were seen in 3 tumors. The epithelial cells of these glands showed large amounts of vitamin A fluorescence.

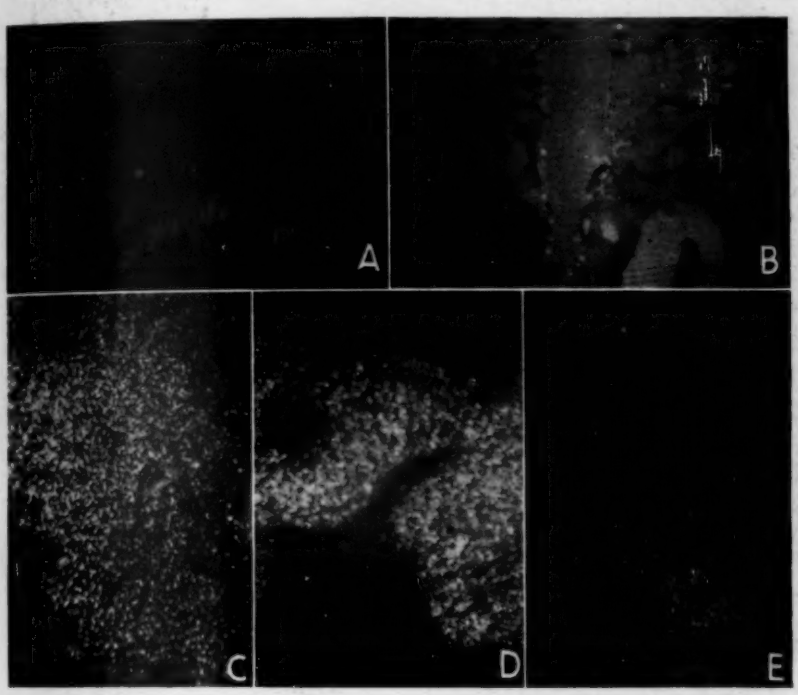

Fig. 2.—Fluorescence photomicrographs. *A*, hypernephroma with vitamin A fluorescence in ringlike distribution around necrotic areas. *B*, malignant teratoma of the ovary, showing vitamin A fluorescence in epithelial cells lining a cyst. *C*, granulosa cell tumor in which vitamin A fluorescence is imparted by medium-sized droplets in the granulosa cells. *D*, granulosa cell tumor in which the distribution of the vitamin A fluorescence resembles that of a corpus luteum. *E*, granulosa cell tumor in which vitamin A fluorescence is imparted by medium-sized droplets in the granulosa cells and by large droplets in the scattered theca cells. The fluorescence of the latter is more dim than that of the former.

(*d*) Five of the tumors revealed in some areas papillary structures. Here, too, small lipoid droplets showing vitamin A fluorescence were present (fig. 1 *E*).

(*e*) In 10 tumors vitamin A fluorescence was present in ring formation (fig. 2 *A*). The rings lined necrotic areas which were rich in

lipoid droplets lying inside or outside the degenerating tumor cells. Some of these droplets were extremely large, owing possibly to coalescence of smaller droplets. The necrotic areas themselves were free of vitamin A. At the edge of these areas, the surrounding intact tumor cells contained large numbers of fat droplets rich in vitamin A fluorescence. This picture suggests absorption of vitamin A from the fat of the necrotic areas.

Two structures of the hypernephroid carcinoma were practically free of vitamin A, namely, the anaplastic carcinoma simplex and the sarcomatous-like forms.

The polymorphous structure of the hypernephroid carcinoma produced a varied distribution of vitamin A fluorescence in the tumor. By examination of different areas in the same tumor and in its metastases, different impressions of the vitamin A content were obtained. The metastases usually showed a sarcomatous-like picture and therefore failed to demonstrate vitamin A, or revealed only traces, which paralleled the fat content. Only a metastasis to bone, a metastasis to the other kidney and a metastasis to a lung revealed considerable amounts of vitamin A fluorescence.

In nearly all of the tumors classified as hypernephroid carcinoma many nonfading fluorescent particles, yellow and brown, were seen in addition to the vitamin A fluorescence. Usually in the presence of vitamin A fluorescence anisotropic lipoids were also observed.

Four tumors of definite renal origin were examined. Three diagnosed as benign cortical adenoma of the kidney showed the characteristic tubular structure with many anisotropic droplets in the epithelial cells, but no vitamin A fluorescence was demonstrated. The fourth was a mucus-producing tumor of the renal pelvis with extension into the medulla, diagnosed carcinoma. It likewise did not show vitamin A fluorescence in the cytoplasm of the tumor cells.

COMMENT

Only certain well defined groups of tumors yield vitamin A fluorescence whereas most of the tumors are free. The finding of vitamin A in approximately one third of the benign or malignant tumors studied indicates that its presence is not connected with tumor formation itself. Local presence of vitamin A does not prevent tumor growth, nor is it necessary for stimulating tumor formation.

The presence of vitamin A in certain groups of tumors is not explained by a relation of vitamin A to tumor formation, but is due to the presence of vitamin A in the parent tissues from which these tumors originate. Usually the distribution of the vitamin A fluorescence simulates that of the parent tissue. In the fluorescence micro-

scopic examination the similarity between the parent tissue and the tumor may be much more obvious than it is in visible light. Other fluorescing particles and the distribution of lipoids, as demonstrated by phosphin 3R, may help in the detection of the similarity.

Vitamin A fluorescence and the presence of fats do not necessarily run parallel, especially in the areas of regressive changes. This fact strongly suggests a specific solution of vitamin A in certain fat bodies. The presence of vitamin A in a tumor does not depend on the nutritional status of the patient nor on the vitamin A stores of the parent tissue. An adrenal with exhausted vitamin A depots may contain a cortical adenoma extremely rich in vitamin A. Vitamin A in tumors is apparently less available for immediate use, quite analogous to lipoma fat in cases of cachexia.

The presence of vitamin A in the solid types of ovarian tumors is significant, since it facilitates the tracing back of some of the tumors to the cells in the normal ovaries which the tumor cells resemble. The distribution of vitamin A may be helpful in the classification of ovarian tumors. The normal ovary except for the ova develops, according to Fischel's [16] hypothesis, from a mesenchymatous tissue. According to recent views, one is inclined to discard the sharp differentiation between mesenchymatous tissue and the germinal surface epithelium. The latter is considered as mesothelium; both mesenchyma and mesothelium are of one entity, the gonadoblastema, which may give rise to the testicle or the ovary.[17] Tissue derived from the gonadoblastema may acquire the property of storing vitamin A in different distributions, as in Leydig and Sertoli cells of the testicle, the granulosa and theca cells, and the cortical stroma of the ovary. Tumors arising from this tissue or from undifferentiated remnants [14] show eventually the same property for carrying vitamin A fluorescence.

Tumors which supposedly arise from the gonadoblastema cells may contain vitamin A independent of their differentiation. The vitamin A fluorescence may be found in the feminizing and masculinizing tumors or in the neutral dysgerminoma. The fluorescence microscopic picture of the dysgerminoma of the testicle and that of the same type of tumor of the ovary show a striking similarity. This supports Meyer's opinion as to the identity of these tumors in both gonads.

The feminizing tumors morphologically show more or less similarity to the structures of the corpus luteum in the fluorescence microscopic examination. Meyer pointed out that such a similarity does not indicate that these tumors have their origin in corpora lutea but suggests parallel morphologic differentiations. This parallelism holds for granu-

16. Fischel, A.: Ztschr. f. Anat. u. Entwcklngsgesch. **92**:34, 1930.
17. Grünwald, P.: Ztschr. f. Anat. u. Entwcklngsgesch. **105**:920, 1936.

losa and theca cell tumors and the usual combination of both, which is more apparent under the fluorescence microscope.

Xanthofibroma is, according to our findings, different from the theca cell tumor. It originates from the "active" ovarian stroma of the cortex. One specimen showed a transitional stage between tumor and hyperplasia of this "active" stroma. Apparently, the functional stage of the cortical stroma is responsible for fibroma free of vitamin A and fat or moderately luteinized xanthofibroma.

Two possible explanations can be offered for the presence of vitamin A fluorescence in the hypernephroid tumors: 1. There is a nonspecific presence of vitamin A as seen in tumors containing plant cells. 2. The tumor has its origin in a parent tissue showing vitamin A fluorescence. Many factors appear to support the latter explanation if the adrenal cortex is considered as the parent tissue. Vitamin A usually appears in the hypernephroid tumors in combination with double refractile bodies. This is normally seen in the adrenal cortex. The fluorescence microscopic examination reveals a similarity of some of the structures in the hypernephroid carcinoma to the normal cortical structures in the adrenal gland, and especially to the benign type of cortical adenoma of the adrenal gland. Even the papillary structures, which have been considered as proof of a renal origin of these tumors, in our series were rich in vitamin A fluorescence. The support of the theory of adrenal rest origin of the hypernephroid carcinoma is enhanced by absence of vitamin A fluorescence from the normal kidney; the distribution of the fluorescence in the nephritic kidney differs completely from that in the hypernephroid carcinoma. The tubular adenoma of the kidney is free of vitamin A, despite the presence of many isotropic and anisotropic lipoids.

The examination by fluorescence microscopy supports the original hypothesis of Grawitz that these tumors originate from adrenal rests and justifies the term "hypernephroma." This theory of an adrenal origin of hypernephromas, supported by some investigators,[18] is not accepted by the majority of the investigators,[19] who have expressed a belief in a renal origin of the so-called hypernephroid carcinoma. Ewing [20] assumed that a small number of renal tumors originate from

18. Wade, H.: Tr. Am. A. Genito-Urin. Surgeons **25**:385, 1932. Gottesman, J. M.; Perla, D., and Elsom, J.: Arch. Surg. **24**:722, 1932.

19. Stoerck, O.: Verhandl. d. deutsch. path. Gesellsch. **12**:123, 1908. Beer, E.: Surg., Gynec. & Obst. **65**:433, 1937. Young, H. H., and Davis, D. M.: Young's Practice of Urology, Philadelphia, W. B. Saunders Company, 1926, vol. 1. Boyd, W.: Surgical Pathology, ed. 3, Philadelphia, Lea & Febiger, 1933. Cabot, H.: Modern Urology, ed. 3, ibid., 1936.

20. Ewing, J.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1940.

adrenal rests. A specific adrenal lipase is present in the blood of patients with adrenal gland diseases and with hypernephroma; it is absent in other renal tumors.[21]

A similarity was noted between normal lactating breast and tubular adenoma of the breast. Only in a few cases was vitamin A fluorescence found in tumors arising from a parent tissue that did not carry vitamin A; i. e., it was found in plant cells in some specimens of carcinoma of the prostate, breast, skin or gastrointestinal tract, and in a few rare tumors. No explanation can be offered as yet regarding these variables.

We believe that the microscopic demonstration of vitamin A is superior to the chemical assay, since in the latter an exact localization is not possible and since necrotic and other regressive areas are included in the weight of the organ.[22]

SUMMARY

With the fluorescence microscope, 219 tumors were examined for vitamin A fluorescence which was encountered only in certain groups of tumors. The findings suggest that the presence or absence of vitamin A does not influence tumor formation.

Vitamin A fluorescence is seen in tumors originating from a parent tissue normally containing vitamin A. The presence and distribution of vitamin A fluorescence help to determine the origin of some tumors, e. g., ovarian tumors.

The fluorescence microscopic picture suggests an adrenal origin of hypernephroma.

The visualization of vitamin A in tumors further suggests an important histologic method for the study of the histogenesis of tumors.

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21. Jorns, G.: Arch. f. klin. Chir. **172**:781, 1933.

22. Some of the tumors studied were also examined chemically with Dr. I. Kraus. Invariably, vitamin A was demonstrated by means of the antimony-trichloride reaction (Carr-Price) whenever vitamin A fluorescence was observed histologically. The nonsaponifiable lipoid fraction of these tumors containing vitamin A showed the characteristic vitamin A fluorescence.

Case Reports

UNUSUAL LOCATIONS OF CARCINOID TUMORS

CHARLES T. ASHWORTH, M.D., AND STUART A. WALLACE, M.D., DALLAS, TEXAS

The carcinoid tumor is known to occur frequently in the appendix and is not considered uncommon in the ileum. On the other hand, reports of its occurrence in other locations are rare. The other locations in which carcinoids have been found are: colon, diverticula, stomach, gallbladder and mesentery. A review of the literature reveals 25 carcinoids in such locations. To these are herein added 3 more, observed in the Baylor University Hospital. Data on these 28 recorded carcinoid tumors are given in the accompanying table.

An analysis of this table reveals the colon to be the most frequent site of carcinoid (11 cases) outside of the appendix and small intestine. In 5 of these 11 cases the tumor arose from the rectum;[1] in 2, from the cecum,[2] and in 2, from the sigmoid colon;[3] in 2 cases[4] we were unable to determine from which portion of the colon the tumor arose. The next most frequent site of occurrence is the diverticulum; 7 cases in which this site was involved have been reported; in 6 of these the site was Meckel's diverticulum,[5] while in the seventh case[6] the type of diverticulum was not mentioned. Next in order of frequency as a site of carcinoid are the stomach (6 cases[7]), the gallbladder (2 cases[8]) and the mesentery (2 cases[9]). A classification of the tumors according to age and sex of patients shows no distinctive characteristics separate

From the Department of Pathology, Baylor University College of Medicine.

1. (*a*) Saltykow, S.: Beitr. z. path. Anat. u. z. allg. Path. **54**:559, 1912. (*b*) Siburg, F.: Frankfurt. Ztschr. f. Path. **37**:254, 1929. (*c*) Brunschwig, A.: J. A. M. A. **100**:1171, 1933. (*d*) Humphreys, E. M.: Am. J. Cancer **22**:765, 1934.

2. (*a*) Raiford, T. S.: Am. J. Cancer **18**:803, 1933. (*b*) Wyatt, T. E.: Ann. Surg. **107**:260, 1938.

3. Saltykow.[1a] Raiford.[2a]

4. (*a*) Kaufmann, E.: Lehrbuch der speziellen pathologischen Anatomie, ed. 9-10, Berlin, Walter de Gruyter & Co., 1931, vol. 1, p. 763. (*b*) Corten: Centralbl. f. allg. Path. u. path. Anat. **56**:364, 1933.

5. (*a*) Stewart, M. J., and Taylor, A. L.: J. Path. & Bact. **29**:135, 1926. (*b*) Hertzog, A. J., and Carlson, L. A.: Arch. Path. **20**:587, 1935. (*c*) Price, I.: Brit. J. Surg. **23**:30, 1936. (*d*) Collins, D. C.; Collins, F. K., and Andrews, V. L.: Am. J. Surg. **40**:454, 1938.

6. Thibaudeau, A. A., and Burke, E. M.: J. Cancer Research **13**:73, 1929.

7. (*a*) Bailey, O. T.: Arch. Path. **18**:843, 1934. (*b*) Pettinari, V.: Arch. ital. di chir. **40**:695, 1935. (*c*) Porter, J. E., and Whelan, C. S.: Am. J. Cancer **36**:343, 1939. (*d*) Masson, P., and Martin, J. F.: Bull. Assoc. franç. p. l'étude du cancer **17**:139, 1928. Raiford.[2a]

8. Joel, W.: Centralbl. f. allg. Path. u. path. Anat. **46**:1, 1929. Porter and Whelan.[7c]

9. Zarakovsky, M. P.: Vestnik khir. **24**:91, 1931.

from those noted by classification according to the more common locations. In 9 of the reported cases the symptoms were dependent on the location of the local growth or on the presence of metastases. Most of the tumors were symptomless and were incidental findings. Seven of the 28 tumors were known to have metastasized.

Data on Twenty-Eight Recorded Carcinoid Tumors Arising in Unusual Locations

No.	Authors	Location	Metastasis	Patient		Size	Symptoms
				Age	Sex		
1	Masson and Martin [7d]	Stomach	Yes				
2	Porter and Whelan [7c]	Stomach	No	60	F	.....	Incidental finding
3	Porter and Whelan [7c]	Stomach	No	40	F	.....	Incidental finding
4	Bailey [7a]	Stomach	No	66	F	13 mm.	Incidental finding
5	Raiford [2a]	Stomach	Yes	55	M	.....	Indigestion
6	Pettinari [7b]	Stomach	No	47	M	3 cm.	Pyloric obstruction
7	Porter and Whelan [7c]	Gallbladder	No	66	F	.....	Incidental finding
8	Joel [6]	Gallbladder					
9	Hertzog and Carlson [5b]	Meckel's diverticulum	No	54	M	4 mm.	Not stated
10	Hertzog and Carlson [5b]	Meckel's diverticulum	No	58	M	3 mm.	Not stated
11	Price [5c]	Meckel's diverticulum	No	54	F	1.5 cm.	Acute diverticulitis
12	Collins, Collins and Andrews [5d]	Meckel's diverticulum	No	56	M	6 mm.	Cramping pain in right lower quadrant of abdomen
13	Stewart and Taylor [5a]	Meckel's diverticulum	No	54	M	Size of pea	Incidental finding
14	Thibaudeau and Burke [6]	Diverticulum	No	37	M	.....	Not stated
15	Wyatt [2b]	Cecum	Yes	55	M	6 cm.	Incidental finding
16	Saltykow [1a]	Rectum	No				
17	Saltykow [1a]	Sigmoid	No				
18	Brunschwig [1c]	Rectum	No	63	M	Small nodule	Incidental finding
19	Siburg [1b]	Rectum	Yes	71	M	Pea	Abdominal pain and mass
20	Kaufmann [4a]	Colon	Yes				
21	Humphreys [1d]	Rectum	No	28	M	7 mm.	Not stated
22	Raiford [2a]	Sigmoid	Yes	53	M	4 cm.	Abdominal mass
23	Raiford [2a]	Cecum	Yes	46	F	.....	Abdominal pain and mass
24	Corten [4b]	Colon					
25	Zarakovsky [9]	Mesentery	No	30	F	Orange	Abdominal mass
26	Authors' case	Rectum	No	31	F	1.5 cm.	Blood in stools
27	Authors' case	Mesentery	No	74	M	2 cm.	Incidental finding
28	Authors' case	Meckel's diverticulum	No	46	M	8 mm.	Incidental finding

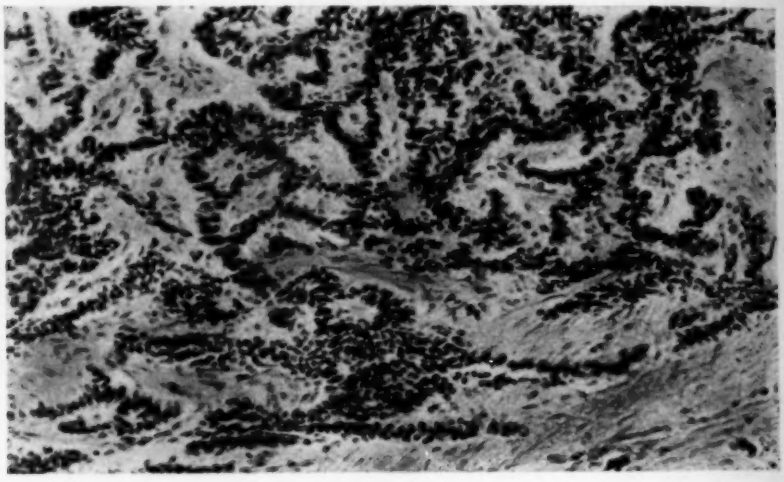
REPORT OF CASES

CASE 1.—A white woman aged 31 complained of the presence of blood in the stools and constipation, each of one year's duration. Proctoscopic examination revealed a small projecting mass high in the rectum. A one stage abdominoperineal resection of the rectosigmoid and rectum was carried out, and the patient recovered satisfactorily.

The resected portions revealed a polypoid mass measuring 0.7 cm. in diameter and composed of grayish red firm tissue. The mass was situated 10 cm. above

the dentate line. The tissue cut with considerable resistance. Immediately adjacent to the mass was an ulcerated area of mucosa, measuring 2.5 by 1.5 cm. There was no induration of the intestinal wall at the base of the tumor or of the ulcer. Careful search revealed no enlarged lymph nodes.

The microscopic sections showed colon with intact glands containing an excess of mucus and lined by epithelium with small uniform nuclei, basally situated. A tumor in the muscular wall was composed of many small cells staining deep blue, with oval or elongated nuclei (figure). These formed narrow bands and strands which appeared to anastomose widely with each other. The nuclei possessed finely granular chromatin. There was a tendency toward palisade arrangement, and in places pseudorosettes were formed. The tumor cells produced no secretion. Mitotic figures were absent. The stroma was composed of smooth muscle and loosely arranged connective tissue, with a few small vessels. An occasional group of tumor cells extended into the mucosa. Also there was extension into the tissue



Carcinoid of the rectum (case 1).

farthest from the lumen. In one region was an area of inflammation with granulation tissue, apparently the base of the ulcer. Tissue was not available for silver staining.

The diagnosis was carcinoid tumor of the rectum.

CASE 2.—A 74 year old white man was admitted with urinary difficulties due to prostatic hypertrophy. Following a transurethral prostatectomy, anasarca and uremia developed and twelve days after admission the man died. Autopsy revealed mainly cortical abscesses of both kidneys and hypostatic pneumonia. An incidental finding was a single yellowish gray nodule in the mesentery of the ileum, measuring 2 by 2 by 1.5 cm. This nodule was somewhat closer to the ileum than to the base of the mesentery. The ileum itself revealed no tumor or mucosal ulceration.

Sections revealed the typical microscopic appearance of carcinoid. The tumor cells were uniform in appearance, with round or slightly oval nuclei, in which the chromatin was abundant and in small clumps. The cytoplasm was light reddish

purple and was small in amount except at the periphery of the tumor masses and also about small blood vessels, where there was a tendency of the cells to line up in a fairly regular row; here the cytoplasm was relatively abundant. Mitotic figures were absent. The tumor cells formed compact small and large masses, through which an occasional small vessel extended. The supporting tissue was composed of mature fibrous connective tissue. In one region there appeared to be invasion of adjacent tissue, although in most areas the tumor was surrounded by a fibrous capsule. Silver-reducing granules were demonstrated in the tumor cells.

The diagnosis was carcinoid tumor of the mesentery of the small intestine.

CASE 3.—A 46 year old white man was admitted in a stuporous condition, which was at first thought to be due to a tumor of the brain but later was found to be due to an atypical form of tuberculous meningitis. An incidental finding at autopsy was Meckel's diverticulum. The anomaly measured 3.5 by 1.5 cm. On opening the diverticulum, a nodule was seen very near the tip, protruding into the lumen, over which the mucosa was intact. The nodule measured 8 mm. in greatest dimension, and on section it was seen to be composed of grayish yellow firm tissue. An imperfect capsule was present.

Sections of the diverticulum revealed mucosa, muscularis mucosae and muscular wall quite typical of small intestine but with excess mucus in the glands and an apparent increase of mucus-secreting cells. There was a tumor of carcinoid type in the wall of the diverticulum, covered by intestinal mucosa. There were many small masses and a few large masses of tumor cells. These were separated by a connective tissue stroma of mature type. The tumor cells had round or occasionally slightly oval nuclei with abundant chromatin in small clumps. Occasional nuclei were large and hyperchromatic. No mitotic figures were present. The cytoplasm was moderate in amount, reddish blue and nongranular. At the periphery of the tumor groups was seen a palisade arrangement of cells with abundant cytoplasm. In the center of a few tumor groups were occasional small spaces about which the cells were arranged as they were about the periphery. The tumor extended into the mucosa through the muscularis mucosae, and in the opposite direction it extended deeper between the muscle bundles. Formaldehyde-fixed tissue was subjected to silver staining, but argentaffin granules were not demonstrated.

The diagnosis was carcinoid tumor of Meckel's diverticulum.

COMMENT

The present consensus is that carcinoid tumors arise from the Kultschitzky cells of the intestinal mucosa (Masson [10]). In accord with this opinion, it is necessary to account for the occurrence of carcinoid tumors in unusual locations. When they are found in diverticula or in the rectum, they may readily be explained, since argentaffin cells occur normally in these locations (Pessin [11]). However, the appearance of primary carcinoids in the mesentery is somewhat more difficult to understand. With regard to this, Masson [10b] stated that the argentaffin cells originate directly from the endoderm of the alimentary canal and under some circumstances migrate to the submucosa. Such an

10. (*a*) Gosset, A., and Masson, P.: Presse méd. **25**:237, 1914. (*b*) Masson, P.: Am. J. Path. **4**:181, 1928; (*c*) **6**:499, 1930.

11. Pessin, S. B.: Arch. Path. **11**:171, 1931.

origin of these cells does not account for the rare carcinoid of the mesentery. In contrast to this, Danisch [12] and Lewis and Geschickter [13] stated that the argentaffin cells are derived from the neurectoderm and migrate first to the celiac ganglions and thence along the courses of nerves to the intestinal wall, finally coming to rest in the mucosa. Argentaffin cells may remain behind, along one of these courses, and then give rise to carcinoid tumors, which would be mesenteric in location.

Generally, carcinoids present a characteristic histologic appearance (cases 2 and 3). Occasionally, however, as in case 1, a slightly different morphologic appearance presents itself. In this instance, the tumor had a closer resemblance to basal cell carcinoma and to certain types of adenoma of the lung. Some [14] have chosen to classify tumors of the latter description as carcinoids, because of this similarity.

Carcinoid tumors are now generally recognized to be potentially malignant, though Forbus [15] originally considered them to be benign. Wyatt [2b] stated that 22.5 per cent of recorded carcinoids of the small intestine were malignant. This compares with 25 per cent for carcinoids in unusual locations. Nodules in the mesentery, which are usually not in lymph nodes (Bailey [7a]), are considered to be the most common form of metastasis. That such nodules may, in some cases, actually represent separate primary carcinoids is suggested from the facts (1) that carcinoids naturally tend to be multiple, (2) primary mesenteric carcinoids are now known to occur and (3) such nodules are often apparently nonprogressive and otherwise innocuous.

SUMMARY

A review of the literature reveals reports of 25 carcinoid tumors in unusual locations, i. e., in sites other than the appendix and small intestine.

Three additional carcinoids are reported: one in Meckel's diverticulum, one in the colon and one in the mesentery of the small intestine.

Of the recorded carcinoids in unusual locations, 25 per cent have metastasized. This is in close agreement with the number of carcinoids of the small intestine which metastasize, namely, 22.5 per cent.

12. Danisch, F.: Beitr. z. path. Anat. u. z. allg. Path. **72**:687, 1923.
13. Lewis, D., and Geschickter, C. F.: Arch. Surg. **28**:16, 1934.
14. Hamperl, H.: Virchows Arch. f. path. Anat. **300**:46, 1937.
15. Forbus, W. D.: Bull. Johns Hopkins Hosp. **37**:130, 1925.

HYPOGLYCEMIA FROM METASTASIZING INSULAR CARCINOMA OF ABERRANT PANCREATIC TISSUE IN THE LIVER

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The syndrome of spontaneous hypoglycemia due to a metastasizing tumor of the islands of Langerhans was first reported by Wilder and his colleagues,[1] in 1927. Since then 7 cases of carcinoma of the island of Langerhans [2] have been reported, in 3 of which metastasis to other organs occurred.[2b,c,d] It is the purpose of this communication to present clinical and pathologic evidence for another case of metastasizing carcinoma of the islands of Langerhans. An unusual feature of this case was that the tumor arose in aberrant pancreatic tissue located in the liver.

REPORT OF A CASE

A 53 year old Russian Jew was admitted to the Montefiore Hospital for Chronic Diseases on July 9, 1940 and died on August 24. The family and personal history were noncontributory. The patient had had chronic cough associated with a great deal of morning expectoration for thirty years, but these symptoms had been relieved by abstinence from cigarets during the past six months.

In December 1939 the patient experienced spells of profuse sweating between the hours of 3 and 6 a. m. He would awake very thirsty and get complete relief from these attacks by drinking a glass of pineapple juice. He also noted a ravenous morning appetite, frequently associated with mental confusion and faintness and relieved by eating breakfast. During the day he frequently stopped for food for fear of fainting while at work. On the morning of Feb. 7, 1940, he became irrational, fainted, had convulsive movements and lapsed into coma; in this state he was seen by a physician and admitted to the Lincoln Hospital. During his two months' stay at this hospital he frequently had similar attacks during which the blood sugar level was reported as too low to read or in the vicinity of 30 mg. per hundred cubic centimeters. By feeding every two hours he was maintained fairly free from these hypoglycemic shock episodes. He was advised to submit to laparotomy at Lincoln Hospital, but refused.

On April 5 he entered the Presbyterian Hospital. Dr. Allen O. Whipple supplied an abstract of the patient's clinical record there. The following relevant observations were made: Coarse rales were heard at the bases of both lungs, and the hepatic dulness extended about 2 fingerbreadths below the costal margin. The temperature, pulse and respirations were normal. The arterial blood pressure was 136 systolic and 70 diastolic. The basal metabolic rate was + 21 per

From the Laboratory Division, Montefiore Hospital for Chronic Diseases.

1. Wilder, R. M.; Allan, F. N.; Power, M. H., and Robertson, H. E.: J. A. M. A. **89**:348, 1927.

2. (*a*) Thalheimer, W., and Murphy, F. D.: J. A. M. A. **91**:89, 1928. (*b*) Hamdi, H.: Ztschr. f. Krebsforsch. **37**:411, 1932. (*c*) Judd, E. S.; Allan, F. N., and Rynearson, E. H.: J. A. M. A. **101**:99, 1933 (*d*) Judd, E. S.; Faust, L. S., and Dixon, R. K.: West. J. Surg. **42**:555, 1934.

cent. The urine contained albumin (1 to 2 plus) and many leukocytes in clumps; the culture yielded a growth of Bacillus coli. The erythrocyte count was 4,880,000, with a hemoglobin content of 13.8 Gm. per hundred cubic centimeters. The leukocyte count was 10,200, with 67 per cent polymorphonuclear neutrophils. The erythrocyte sedimentation rate was 29 mm. at the end of one hour. The Kline test was negative. Chemical studies of the blood revealed a phosphorus content of 4 mg. per hundred cubic centimeters; phosphatase, 11.5 Bodansky units; calcium, 10.2 mg. per hundred cubic centimeters; nonprotein nitrogen, 25 mg.; bilirubin, approximately 1 mg.; cholesterol, 184 mg.; total serum protein, 6.7 Gm., of which the albumin and globulin contents were 4.0 and 2.7 Gm., respectively. The fasting blood sugar level was 36 mg. An oral dextrose tolerance test showed a rise to 125 mg. and a falling off to shock levels in four hours. A roentgenogram of the chest disclosed a shadow from increased density in the inner portion of the right lower lung field, which was demonstrated by injection of iodized poppy-seed oil 40 per cent to be an area of atelectasis. Roentgen examination gave no suggestion of a tumor in the lungs or in the skeletal structures. Bronchoscopic examination showed normal bronchi.

During the period of hospitalization the patient had many typical episodes of hypoglycemic shock, and these were immediately relieved by intravenous injection of a solution of dextrose. On a regimen of 20 Gm. of carbohydrate every two hours the patient was kept moderately comfortable. The diagnosis was hyperinsulinism due to an adenoma or, possibly, a carcinoma of the islands of Langerhans, and on April 23 an exploratory laparotomy was performed by Dr. Allen O. Whipple, who found that the tumor was not in the pancreas. A good deal of time was consumed in searching for the tumor. Strangely enough, the growth was finally found to occupy the retroperitoneal area between the upper border of the head of the pancreas and the spigelian lobe of the liver.

It was a large tumor, measuring 10 by 6 by 6 cm. In its upper limits it appeared to infiltrate the liver, but the lower limits did not seem continuous with the pancreas. It was irremovable because it was pressing on the portal vein and infiltrating the liver. As a result of the pressure on the portal vein, the portal radicles showed considerable distention and engorgement; this was first noted on the lower border of the pancreas when attempts were made to mobilize the pancreas for palpation. Here a branch of the junction of the inferior mesenteric with the splenic vein caused a great deal of bleeding, which was difficult to control. Other small radicles bled easily. The whole operation was unusually difficult because of the obesity of the patient and the portal engorgement. "The condition being inoperable, a specimen was taken for biopsy, and the abdomen closed."

The postoperative course was stormy for two weeks. The patient had pulmonary edema, which was relieved by phlebotomy. On the fifth day after operation it was necessary to resuture the operative wound, which had disrupted. Subsequently, roentgenographic signs of pneumonia in the right lung were noted, and intravenous injection of sodium sulfapyridine (2-[paraaminobenzenesulfonamido] pyridine) was instituted. During this time the blood sugar was well controlled by parenteral administration of fluids. The patient was doing moderately well when, on the twenty-fifth day after operation, hypoglycemic shock recurred. The carbohydrate intake was increased, and feedings were spaced more frequently, but shock episodes continued to increase in number and severity until it became necessary to administer dextrose intravenously, every twenty-four to forty-eight hours. His average carbohydrate intake was 350 Gm. daily, and he was fed

hourly. A short course of radiotherapy was unsuccessful in relieving symptoms. He was discharged from the Presbyterian Hospital on June 19 and reentered Lincoln Hospital, where he remained with condition unchanged until his transfer to the Montefiore Hospital for Chronic Diseases on July 9.

On admission the patient was moderately obese. The relevant physical findings were the same as noted previously with the addition of the laparotomy scar. The erythrocyte count was 4,670,000, with a hemoglobin value of 92 per cent. The leukocyte count was 13,050, with 76 per cent polymorphonuclear neutrophils. The blood sugar one hour after drinking a glass of orange juice was, on one occasion, 25 and on another 39 mg. per hundred cubic centimeters. With an initial blood sugar level of 25 mg., an oral dextrose tolerance test was made. A maximum level of 69 mg. was reached after fifteen minutes, and at the end of one hour the level was 23 mg. The blood urea nitrogen was 13.4 mg. The basal metabolic rate was + 18 per cent. Two electrocardiograms showed the heart normal except for sinus tachycardia.

Continuous intravenous administration of 5 per cent dextrose was required to abate hypoglycemic symptoms, and during the last month of life hourly subcutaneous administration of epinephrine was frequently required, in addition, to control these symptoms. On July 18 there were physical and roentgenographic signs of a pneumonic process in the lower half of the right lung, associated with an irregular fever. On July 25 a firm, slightly tender nodule was found in the skin over the outer third of the right clavicle, and the liver had enlarged so that its edge was 4 to 5 fingerbreadths below the costal margin. Other than the progression in the hypoglycemic episodes there were no clinical changes until death on August 24. The pulmonary signs persisted. A preterminal shift to a diet high in protein and fat and low in carbohydrate, supplemented with orange juice and epinephrine as needed, failed to alter the clinical picture.

Autopsy (eight hours after death).—The body was that of a well developed and well nourished adult white man measuring 175 cm. in height. A firm nodule, 1 cm. in diameter, was noted beneath the skin over the outer third of the right clavicle. It moved freely in the subcutaneous tissue and presented irregular grayish white fibers studded with yellowish specks. There was no icterus.

There was no external lymphadenopathy. One small tumor nodule was found in the mesentery, which was otherwise rich in fat. The retroperitoneal lymph nodes were all much enlarged, especially those around the renal arteries and the inferior vena cava. Here they measured up to 5 by 2 cm. in greatest dimensions and were completely replaced by a grayish white firm tumor-like tissue containing many streaks and foci of yellowish discoloration. The right hilar and portal lymph nodes were similarly involved.

The right lung on its anterior surface and the left lung on all surfaces showed an old fibrous obliterative pleuritis. Both lungs were heavy and voluminous, the heaviest portion being the upper lobe of the right lung. The lower two thirds of this lobe was hard, grayish and yellowish white, and irregularly nodular. This portion was composed of confluent grayish white nodules, rich in contracted fibrous tissue and studded with yellowish foci. All radicles of the pulmonary artery going to this lobe were compressed, stenotic and completely surrounded by grayish and yellowish white tumor-like tissue. A typical wedge-shaped hemorrhagic infarct, 3.5 cm. in greatest width, was found at the junction of the consolidated lower two thirds of the lobe with the upper one third. The middle lobe of the right lung was not a separate definable lobe; it seemed to be incorporated within the consolidated portion of the upper lobe. The lower lobe of the right lung and

the left lung were large, diffusely emphysematous with focal subpleural blebs, moderately congested and edematous. All bronchi were diffusely dilated and filled with purulent material. The tracheobronchial lymph nodes were enlarged, fibrotic and anthracotic and showed small grayish and white foci. The right hilar nodes were tumorous, as mentioned.

The heart was hypertrophied and weighed 450 Gm.

The liver weighed 3,400 Gm. and measured 33 by 22 by 15 cm. The anterior surface was smooth, glistening and slate brown. The inferior border was attached by fibrous tissue to the transverse colon and to the abdominal muscles along the line of the healed operative incision. Similar fibrous adhesions loosely bound the inferior portion of the posterior surface of the liver to the stomach and duodenum. Beneath these adhesions this portion of the liver showed no abnormalities. The upper portion of the posterior surface of the liver had two large, essentially regular inherent firm masses. These two masses lay on either side of the channeled inferior vena cava. The medially located mass measured 9 by 6 by 4 cm. and the lateral one 13 by 11 by 5 cm. Their external surfaces were glistening, nodular but smooth, grayish and yellowish white and unattached to any of the surrounding viscera. Serial sections through the liver revealed that both these tumor masses were direct continuous protrusions of a single large tumor mass located within the central portion of the liver. The central tumor area measured 17 by 11 cm. in greatest dimensions and was well demarcated but not encapsulated. The superior, anterior and inferior surfaces of the tumor were surrounded by a considerable amount of apparently normal hepatic tissue, which was thinnest anteriorly. The tumor mass was firm and cut with marked grittiness to present a grayish white structure with areas of dense, contracted scar tissue and areas of chalky, yellow necrotic matter. The tumor tissue roughly approximated half the total volume of the liver. The portal vein was caught within the tumor mass on the posterior surface of the liver and was considerably compressed; near its main subdivision were several large mural thrombi incompletely occluding the lumen.

Each of the adrenals weighed 9 Gm. Two small tumor nodules were found in the left adrenal and one larger nodule, 1 by 1 cm., in the right.

The pancreas weighed 45 Gm. and was 14.5 cm. in length and 3 cm. in width. It lay in the normal position and was of normal appearance except that its head was not wider than the rest of the organ. Serial sections failed to reveal any gross abnormality.

The spleen weighed 500 Gm. and measured 18 by 10 by 7 cm. The superior pole was densely adherent to the diaphragm and contained a 7 by 7 cm. cystic area. On section the cyst was filled with a turbid yellowish white fluid containing friable bits of tissue. The cyst was lined by a friable grayish and yellowish white network; its wall measured 0.5 cm. The remainder of the spleen was soft, mushy and purplish red. The splenic vessels showed no changes.

The kidneys were enlarged; each weighed 270 Gm. Tumor nodules were found in the adherent perinephric fat, especially in the hilar areas. Each kidney contained from six to ten tumor nodules, which measured up to 4.5 cm. in greatest dimension and replaced approximately half the parenchyma.

The lumbar vertebrae were normal.

Microscopic Examination.—The pancreatic acini and ducts were normal. The islands were normal in structure and for the most part normal in size; an occasional island was somewhat enlarged. The interlobular and periductal connective tissue was moderately abundant.

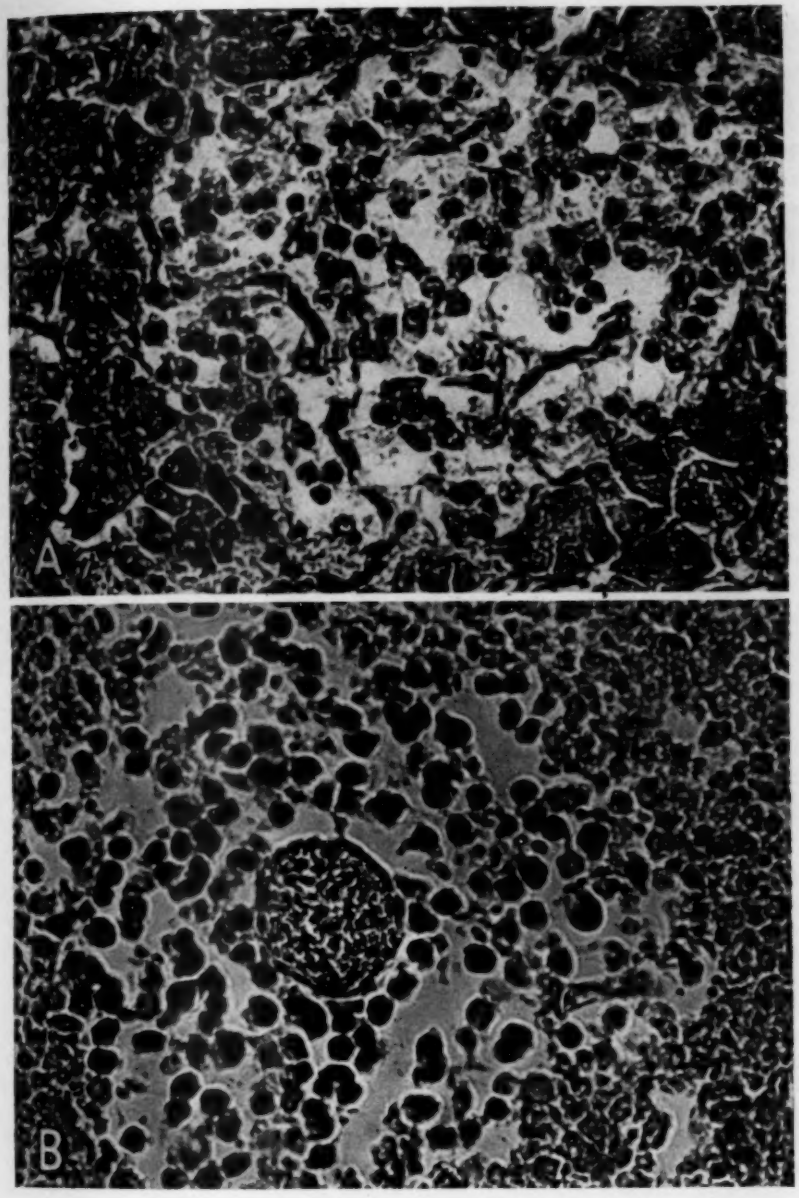

Fig. 1.—*A*, section through pancreas showing a representative normal island of Langerhans; × 480. *B*, section through the protruding tumor mass in the liver above the porta hepatis showing necrotic tumor with a nest of viable tumor cells; note the resemblance of the latter to an island of Langerhans; × 480.

The tumor in the liver was fairly well demarcated but not encapsulated and was composed of large irregular sheets or small nests of cells lying close to a rich capillary network in a delicate connective tissue stroma. The cells were large and of irregular shape. Within the larger masses they were closely packed, while in many of the small nests or islands of tumor they were more loosely arranged. Their cytoplasm was pale, usually neutrophilic, finely granular and rather abundant. Their nuclei were large, vesicular and hyperchromatic; each had a clearly defined nucleolus and a fine network of chromatin. An occasional nucleus was vacuolated and markedly swollen. Mitotic figures were moderately numerous, and multinucleated giant cells were also present. The tumor cells, particularly those within the small nests of tumor, bore a striking resemblance to the cells of the islands of Langerhans. Within the tumor mass were large areas

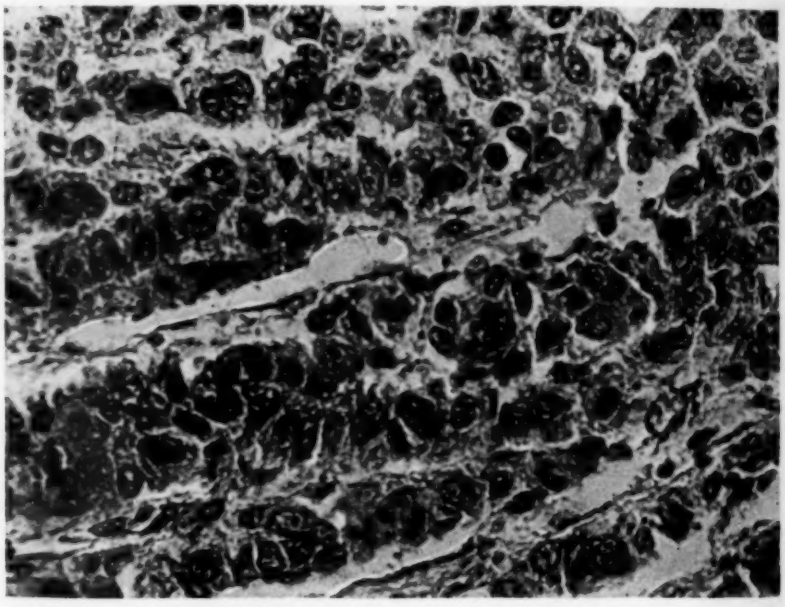

Fig. 2.—Section through tumor within the liver, showing the arrangement of tumor cells in solid sheets; × 480.

of necrosis, bands of fibrous tissue and thin layers of intact hepatic cords. Tumor cells were found within a large radicle of the portal vein, and several of the blood vessels were filled with bland thrombi.

The nontumorous portion of the liver showed evidences of moderate parenchymatous degeneration and slight vacuolation. The sinusoids were slightly engorged with blood cells, and occasionally they contained small clumps of three or four tumor cells. Within the portal canals fibrous tissue was slightly excessive and associated with an increase in lymphocytes.

The same type of richly vascularized and partially necrotic tumor was found in the consolidated upper lobe of the right lung, where there was also an area of hemorrhagic infarction surrounded by patches of bronchopneumonia. The other lobes showed small peribronchial tumor foci, chronic purulent bronchitis, fibrosis

and emphysema. The right hilar lymph node was completely replaced by extensively necrotic tumor.

The heart had a small tumor focus in the subendocardial portion of one of the trabeculae carneae of the right ventricle.

The wall of the vena cava inferior was invaginated by masses of tumor cells which extended from without inward but did not break through the intimal lining.

The cystic portion of the spleen had a lining of tumor tissue rich in fibrous and necrotic areas. Small tumor thrombi plugged the sinusoids.

Each adrenal contained a sharply defined tumor mass, which was but slightly necrotic.

The kidneys showed many areas of tumor invasion of the cortex and medulla. The tumor was only slightly necrotic and was composed of large sheets of closely packed cells. Tumor cells were found within the larger venous radicles. The arterioles were somewhat sclerosed and stenosed.

The subcutaneous nodule over the right clavicle contained many small intact metastatic tumor foci.

The prostate gland revealed adenomatous and fibromuscular hyperplasia.

Experiment on Rabbits to Determine Source of Hypoglycemia in Case Reported

Source of Extract	Amount Injected, Gm.	Rabbit's Fasting Blood Sugar, Mg. in 100 Cc.	Blood Sugar After Injection of Extract, Mg. in 100 Cc.	
			1 Hour	2 Hours
Control pancreas	28	122	51	33
Control liver	75	116	108	111
Control kidney	80	119	115	120
Pancreas of patient	30	103	...	80
Tumor tissue in liver of patient	75	98	...	73
Tumor tissue in liver of patient	500	121	...	71

In the testes spermatogenesis was noticeably diminished. The tubules were small and atrophic, the basement membranes were thickened, and the stroma was focally fibrotic and edematous. The interstitial cells were large, deeply pigmented and normal in number.

Postmortem Chemical Studies (Dr. E. J. Baumann).—The pancreas and the tumor mass within the liver were treated chemically according to the method of Abel and Geiling [3] for the extraction of insulin. Unfortunately this was done approximately forty-eight hours after death and was consequently not of optimum value. The extracts obtained from the pancreas and the partially necrotic tumor mass in the liver were injected subcutaneously into fasting rabbits; the blood sugar levels were then studied for a two hour period. As a control, extracts from equal amounts of nondiseased pancreas, liver and kidney, taken two days after death from an adult cadaver which showed local recurrence of a resected carcinoma of the stomach, were similarly examined (table.)

COMMENT

In view of the fact that no tumor or other pathologic condition was found in the pancreas, the question arises whether the hypoglycemic episodes which characterized the clinical course of this patient's disease

3. Abel, J. J., and Geiling, E. M. K.: J. Pharmacol. & Exper. Therap. **25**:423, 1925.

were due to the elaboration of a hypoglycemic agent by the extrapancreatic tumor or whether the episodes were of endocrine or hepatic origin.

A hepatic origin may be discounted in the light of (1) the very considerable amount of hepatocellular tissue which histologically was within relatively normal limits, (2) the normal bilirubin content of the blood, (3) the normal total serum proteins and normal albumin-globulin ratio, (4) the absence of macrocytosis of the red blood corpuscles, (5) the excellent, often repeated therapeutic response to frequently administered epinephrine, which clinically implied the existence of adequate stores of readily mobilizable glycogen within the liver, and (6) the results of the dextrose tolerance tests and the absence of glycosuria.

In hepatic disease with badly impaired glycogen function the dextrose tolerance curve is of the high plateau type and resembles the diabetic curve, except that the fasting blood sugar content is abnormally low or normal. This patient, who had been given a high carbohydrate diet for several months, had, on the contrary, a dextrose tolerance curve with a moderate early rise followed by a return to hypoglycemic shock levels. With progression of the disease a subsequent dextrose tolerance test showed a much lower postprandial elevation, soon followed by a return to hypoglycemic levels. If such change in the dextrose tolerance curve were of primary hepatic origin, it would have been necessary for the hepatocellular damage to be most extensive and diffuse. Neither the autopsy nor the clinical findings, however, indicated such severe diffuse hepatic damage. Nor could evidence be found that would implicate the endocrine system. The adrenal glands had sufficient morphologically intact and normal cortical and medullary tissue to maintain adequate function in spite of inherent focal metastatic lesions. The thyroid and pituitary glands were not examined owing to the limitations imposed on the autopsy, but there was no clinical evidence for hypofunction of these glands or of the adrenal glands to explain the hypoglycemic syndrome. The pancreas, studied grossly and microscopically, failed to show any pathologic changes in the islands of Langerhans. However, an extensive microscopic study of serial sections of the pancreas was not made.

There remains, therefore, the tumor as a possible source of a substance producing the hypoglycemic syndrome. Against the possibility that nonspecific tumor tissue by itself may cause hypoglycemia is the fact that tumor tissue arising outside the endocrine system has never been demonstrated to possess or to liberate substances capable of creating a clinical picture of spontaneous hypoglycemia. On the contrary, Woodward and Fry [4] reported an elevated fasting blood sugar and dextrose tolerance curve in patients with cancer uncomplicated by diabetes. Furthermore, the tumor tissue in this case presented a striking resemblance to the cells of the island of Langerhans and by chemical studies was found to contain a hypoglycemia-producing substance. It is presumed, therefore, that the tumor developed within aberrant pancreatic tissue located within the liver.

While aberrant pancreatic tissue has been found in other viscera, it has never been reported in the human liver. Thorsness [5] stated that in

4. Woodward, G. E., and Fry, E. G.: Biochem. J. **26**:889, 1932.

5. Thorsness, E. T.: Anat. Rec. **77**:319, 1940.

90 per cent of patients with aberrant pancreatic tissue this tissue is found in the foregut. The literature contains no report of a case in which aberrant pancreatic tissue underwent malignant transformation. In this respect, therefore, this case presents an additional interesting feature.

That the hypoglycemic episodes in this case were caused by the malignant tumor is implied also by the parallelism between the development of multiple, widely distributed metastatic lesions and the increasing frequency and severity of the symptoms. The metastatic nodules elaborated additional hypoglycemia-producing substance to the point where only continuous infusion of dextrose and hourly administration of epinephrine could relieve or abate the shock episodes. No doubt the later metastatic lesions were largely responsible for the preterminal clinical picture of severe shock, for the earlier lesions, such as those in the liver, were largely degenerated and necrotic. Endocrine tumors notoriously require a large supply of blood, and when cellular proliferation outdistances the vascular supply, necrosis rapidly sets in. In the absence of metastases such necrosis, if extensive, might result in cessation of the progress of the disease and perhaps even in regression. By the process of elimination supported by a striking morphologic resemblance of the tumor tissue to the cells of the island of Langerhans and a typical clinical syndrome, one reaches the conclusion that the tumor was carcinoma of islands of Langerhans arising in aberrant pancreatic tissue within the liver.

SUMMARY

An unusual case is reported of spontaneous hypoglycemia due to a metastasizing carcinoma of islands of Langerhans arising within aberrant pancreatic tissue in the liver.

General Reviews

CONSTITUTION AND RELATED FACTORS IN RESISTANCE TO TUBERCULOSIS

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PHILADELPHIA

(Concluded from page 162)

INTERNAL SECRETION AND METABOLISM

A determining influence of the glands of internal secretion has been hypothecated by certain writers, who are convinced that inherent constitution is important but who have failed to correlate resistance with the build of the body (Ickert [15]). Because of the differences in mortality in the two sexes at different periods of life, more attention has been devoted to gonadotropins and to estrogens and androgens than to other endocrine principles. The possible effects of gonadotropic, estrogenic and androgenic substances were discussed in the preceding section. The role of other endocrine factors will now be considered.

Hyperthyroidism and Hypothyroidism.—In reviews on the relations of the several internal secretions to the course of tuberculosis Kallos and Kentzler [144] and Lisser [145] laid chief stress on the influence of the thyroid gland. They recalled that early pathologists, including Rokitansky and Virchow, believed an antagonism existed between hyperthyroidism and tuberculosis and that clinicians felt they had confirmed the existence of this supposed antagonism. According to Joll,[146] thyrotoxic patients had little tendency to contract pulmonary tuberculosis. Fishberg [147] considered hyperthyroidism an indication of favorable prognosis in patients with incipient tuberculosis. In contrast, a clinical impression has grown up that patients with hypothyroidism and myxedema are unusually susceptible to tuberculosis. Kallos and Kentzler cited several experiments on the effect of extirpation of the thyroid gland on resistance to tuberculosis. In general, animals deprived of this gland displayed poor resistance. In their own experience they found that guinea pigs with the thyroid intact survived, on the average, twice as long after infection

144. Kallos, P., and Kentzler, J.: Beitr. z. Klin. d. Tuberk. **79**:584, 1932.

145. Lisser, H.: Am. Rev. Tuberc. **29**:249, 1934.

146. Joll, C. A.: Diseases of the Thyroid Gland, St. Louis, C. V. Mosby Company, 1932.

147. Fishberg, M.: Pulmonary Tuberculosis, ed. 4, Philadelphia, Lea & Febiger, 1932.

with 0.1 mg. of virulent tubercle bacilli as did those with the thyroid gland removed.

A relation was noted between basal metabolism, the state of the thyroid gland, allergy and resistance. Kallos and Kentzler agreed with other clinicians that the course of tuberculosis is commonly benign in the presence of high basal metabolism. In their experience the onset of tuberculosis appeared to stimulate the thyroid gland to greater activity. The latter effect acted as a defense mechanism. If it failed to occur, the subsequent course of the disease was not apt to be favorable. In such patients the reaction to tuberculin was feeble. If, however, thyroid extract was administered, tuberculin allergy increased and the clinical course improved.

However, tuberculosis may be progressive in the presence of hyperthyroidism. Vidal and Doré [148] reported the case of a patient with hyperthyroidism and tuberculosis and a basal metabolic rate of + 45 per cent. The tuberculosis pursued a grave course until thyroidectomy was performed, after which good healing occurred. Crile [149] reported 87 cases of thyroidectomy in tuberculous patients with hyperthyroidism and saw improvement in 74 per cent.

Thus there is some conflict in evidence, but the majority of investigators have claimed that hyperthyroidism and increased basal metabolism are associated with good resistance to tuberculosis.

With respect to the relations of other glands of internal secretion to tuberculosis, excluding the pancreas, there is little clinical evidence. Mistal [150] reported a variety of endocrine abnormalities in tuberculous patients, but conclusions can hardly be drawn as to the relations to resistance without data on contact and other factors. Hernandez and Boyer [151] reported 2 cases of adiposogenital dystrophy and tuberculosis. The course of the latter was chronic.

Steinbach [152] obtained evidence that bilateral adrenalectomy lowers the natural resistance of rats to tuberculosis. In his experiments with bovine and avian type bacilli lesions developed in the great majority of animals whose adrenals had been removed and in only half of the normal controls infected with avian bacilli and in none of those infected with bovine bacilli. Perla and Marmorston [62] confirmed these results and found that a strain of rats refractory to infection with human type bacilli could be infected with this strain after adrenalectomy. Additional data following this interesting lead do not appear to have been secured.

148. Vidal, J. A., and Doré, R.: Union méd. du Canada **68**:262, 1939; abstracted, Zentralbl. f. d. ges. Tuberk.-forsch. **50**:615, 1939.

149. Crile, G. W.: Surg., Gynec. & Obst. **58**:272, 1934.

150. Mistal, O. M.: Arch. méd.-chir. de l'app. resp. **11**:189, 1936.

151. Hernandez, I. M., and Boyer, M.: Arch. argent. de tisiol. **14**:336, 1938.

152. Steinbach, M. M.: Proc. Soc. Exper. Biol. & Med. **27**:142, 1929.

Diabetes.—A relation between tuberculosis and diabetes has been recognized since ancient times. Almost all investigators are agreed that diabetes in some way lowers resistance to tuberculosis. The only argument is whether diabetes has as severe an effect on the course of the disease as the early writers believed. The present tendency is to demonstrate that if patients have suitable care the course of tuberculosis need not be unfavorable. It is not possible to review here the large literature on the subject. For a detailed consideration of various phases of the subject the reader is referred to a series of papers by Root.[153]

According to Root, pulmonary tuberculosis is found at necropsy two or three times as frequently in diabetic as in nondiabetic persons in the general population, and the type encountered is apt to be more acute than that usually observed in white persons of corresponding sex and age. He mentioned caseation of the lymph nodes as characteristic, but the protocols of the cases extracted from his own experience do not indicate an excessive trend in this direction. Miliary and generalized tuberculosis and tuberculous meningitis did not appear to Root to occur with unusual frequency in cases of diabetes. There was no evidence of a fundamental lack of resistance, for healed primary tuberculosis and healed lesions of late life were commonly observed in the bodies of diabetic persons. The conclusion drawn was that resistance is normal until diabetes develops. Perla and Marmorston [62] found nothing distinctive in the character of tuberculous lesions in diabetic persons observed at necropsy and stated that the chief characteristic of pulmonary tuberculosis in the presence of diabetes was the rapidity with which it developed.

Root's analysis indicates that tuberculosis occurs far more commonly after the onset of diabetes than before. In 245 cases of combined tuberculosis and diabetes in his experience, the diabetes came first in 85 per cent. Late diagnosis was the rule. In those cases in which the onset was observed there was nothing distinctive about the early stages or the course. The incidence of tuberculosis increased with the duration of the diabetes. In 49 of the 245 cases studied tuberculosis developed after the patient had reached the age of 60 years. Juvenile diabetic patients, however, seemed particularly susceptible, for the incidence of tuberculosis in this group was ten times that in nondiabetic children of the same age.

Root found frequent evidence of a relation between acidosis and coma and the subsequent development of tuberculosis. In a significant percentage of cases the disease developed within three years after recovery from coma. This suggested to Root, as it has to many others, that factors concerned with body chemistry are implicated in the lessened resistance to tuberculosis. As possibly concerned, he mentioned disordered protein and fat metabolism with increased amino acid and

153. Root, H. F.: New England J. Med. **210**:1, 78, 127 and 192, 1934.

glycerol content in the tissues, especially in periods of acidosis. Other authors have laid chief emphasis on the excessive dextrose content of the tissues. In studies he carried out with Bloor [154] a much lower concentration of phospholipid and lipid was found in the lungs of diabetic than in those of nondiabetic persons. On the other hand, a high lipid content is characteristic of the liver, blood and blood vessels in diabetes.

Root felt that in diabetes the cultural conditions must be favorable in some way to the growth of tubercle bacilli and believed that such conditions were created by abnormal fat metabolism. He referred to the work of Long and Vorwald [155] as of possible significance for diabetes. These authors, recognizing the great nutritional value of glycerol for tubercle bacilli in cultures, studied its effect on tubercle bacilli in vivo by feeding or injecting glycerol over a long period into tuberculous rats. The treatment appeared definitely to enhance the extent of the disease, as shown by comparison with nontreated controls. In an attempt to produce the opposite effect, i. e., to lower the content of glycerol in the tissues, they gave tuberculous rats large quantities of free fatty acid, assuming that if free glycerol was present, combination with the excess of fatty acid would occur in accordance with the chemical principle of mass action. In the majority of animals the tuberculosis was much less extensive than in the glycol-treated animals, but the fatty acid treatment itself was harmful to health. Subsequent experiments have yielded similar but less decisive results.[156] The great difficulty in the way of proper experiments of this character is the unavailability of quantitative methods for the determination of glycerol in body fluids.

For other articles dealing with various phases of the clinical and pathologic problems the reader is referred to Wiener and Kavee,[157] King,[158] Ralli and Steinberg,[159] Müller [160] and Fett.[161] The conclusions of these authors are similar to those reached by Root. No significant chemical or other constitutional factors responsible for the severity of tuberculosis in diabetic patients were identified.

Rest [162] studied the course of tuberculosis in Jews, a racial group traditionally prone to diabetes and resistant to tuberculosis. Rest found that the tuberculosis pursued a more favorable course in the nondiabetic than in the diabetic Jew, with more fibrosis and longer duration. In his experience tuberculosis became manifest chiefly in two periods, early

154. Root, H. F., and Bloor, W. R.: Am. Rev. Tuberc. **39**:714, 1939.
155. Long, E. R., and Vorwald, A. J.: Am. Rev. Tuberc. **22**:636, 1930.
156. Long, E. R.: Unpublished data.
157. Wiener, J. J., and Kavee, J.: Am. Rev. Tuberc. **34**:179, 1936.
158. King, S. E.: Quart. Bull., Sea View Hosp. **1**:416, 1936.
159. Ralli, E. P., and Steinberg, I.: J. Lab. & Clin. Med. **23**:581, 1938.
160. Müller, E. M.: Ztschr. f. Tuberk. **80**:281, 1938.
161. Fett, A.: Ztschr. f. Tuberk. **82**:113, 1939.
162. Rest, A.: Am. Rev. Tuberc. **43**:344, 1941.

adult life and middle age. Most deaths occurred between 40 and 60, and the fatal issue was a result as much of the diabetes as of the tuberculosis.

Many authors, recognizing the generally unfavorable course of tuberculosis in the presence of diabetes, have put exceptions on record (for example, Wessler and Hennell [163]). Great improvement in the prognosis of tuberculosis in diabetic patients came with the advent of insulin. The favorable action of the latter is apparently not an effect per se but is a result of the improvement in metabolism that follows administration of insulin (Allen [164]). If hyperglycemia is a factor in promoting the growth of tubercle bacilli in the body—a possibility, since dextrose, like glycerol, favors the growth of tubercle bacilli—insulin might affect resistance to tuberculosis almost directly by reducing the amount of sugar in the blood and body fluids.

Experimental diabetes should prove fruitful for an understanding of the apparent lowering of resistance to tuberculosis. Steinbach has commenced an experimental approach. He, Klein and Deskowitz [165] have inoculated normal and depancreatized dogs with human type tubercle bacilli. In the control animals the disease has been isolated and mild, whereas in the depancreatized dogs it has been invasive and generalized. Similar experiments are said to be in course and to have shown like results thus far.

Other Metabolic Disorders.—Traditionally tuberculosis is mild in the presence of gout. Recently Griesbach and Anton [166] reviewed previous literature and reported new studies on the relation of tuberculosis and uric acid metabolism. Previous results indicated that a purine-rich diet raised the blood uric acid level in healthy persons and in patients with chronic, productive types of tuberculosis of favorable course but failed to do so in patients with acute exudative tuberculosis. In 1939 he reported a second series of cases. In 72 cases in which the patients were subjected to long and exact observation, with at least five blood analyses, a high concentration of uric acid in the blood was regularly associated with a benign course of the disease. In another series of cases, with determinations of blood uric acid on hospital admission, the cases that proved to have a good prognosis averaged a blood concentration of 3.68 mg. of uric acid per hundred cubic centimeters, while the average concentration in those cases in which the course proved unfavorable was 2.76 mg.

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163. Wessler, H., and Hennell, H.: Am. Rev. Tuberc. **27**:47, 1933.
164. Allen, F. M.: Am. Rev. Tuberc. **34**:230, 1936.
165. Steinbach, M. M.; Klein, S. J., and Deskowitz, M.: Am. Rev. Tuberc. **32**:665, 1935.
166. Griesbach, R., and Anton, W.: Beitr. z. Klin. d. Tuberk. **93**:161, 1939.

There is frequent reference to the relation of tuberculosis and general metabolism as it may be affected by climate. Mills [167] related the course of the disease to the climatic conditions and the corresponding level of metabolism. An obvious difficulty in such a study is that of determining the exact time of onset. Account must be taken of the frequent long period of clinical latency and of the uncertainty in a given case whether the disease as found clinically represents fresh exogenous infection or a recrudescence of a long dormant lesion.

NUTRITION

Malnutrition is popularly supposed to predispose to tuberculosis, and feeding in the effort to restore normal weight and strength has been one of the mainstays of treatment since early times. Inasmuch as the loss of adipose tissue is conspicuous in advancing tuberculosis, excessive administration of fat-building foods was common in the past. At present more attention is concentrated on proteins and vitamins. For reviews of the literature the reader is referred to Clausen [168] and Tissue.[169]

Much of the work to be discussed in the following pages was experimental. Some was based on clinical observation. Reference should be made first, however, to deductions drawn from epidemiologic studies of tuberculosis in populations that were in a state of dietary deficiency.

Epidemiologic Studies.—During the World War, 1914 to 1918, a sharp increase in the death rate from tuberculosis occurred throughout Europe. After years of continuous drop, the rate began rising in 1915 and by 1918 had reached a figure in all countries about 25 per cent higher than at the beginning of the war. Wolff [170] has described the privations of the period as "an involuntary mass experiment . . . of more epidemiological importance than endless theorizing on the pathology of tuberculosis." The numerous investigators who analyzed the causes of the increase dealt particularly with nutrition and housing. Since the impairment of the normal food supply and the rise in tuberculosis both preceded the housing shortage and crowding, most writers attributed the increase of tuberculosis to malnutrition. Schröder [171] reviewed the literature of the 1920's in Germany and stated the conclusion of most German writers that crowding was not the primary factor and that, of all the dietary elements, the reduction in protein and fat was most significant. Protein had been reduced to 80-90 Gm. daily and the fat to 90-100 Gm., amounts that Schröder considered insufficient to pre-

167. Mills, C. A.: Am. J. M. Sc. **189**:330, 1935.
168. Clausen, S. W.: Physiol. Rev. **14**:309, 1934.
169. Tissue, K. A.: J. Am. Dietet. A. **16**:313, 1940.
170. Wolff, G.: Human Biol. **10**:106, 1938.
171. Schröder, G.: Beitr. z. Klin. d. Tuberk. **75**:61, 1930.

vent breakdown in patients with slight lesions who would have been safely protected by normal diets. Schröder stated that there was a sufficiency of fresh vegetables and fruit, with their vitamins, throughout the war.

An especially interesting analysis was made by Faber [172] of conditions in Denmark, a country not at war but subjected to influences of sea blockade and high food prices. A rise in the tuberculosis rate much exceeding the rise in the general death rate began almost immediately after the initiation of hostilities. A significant difference between Denmark and surrounding countries became apparent, however, before the end of the war. Whereas in most countries the high incidence of tuberculosis continued up to the end of the war or even for a year or two after it, in Denmark a sharp drop occurred in 1918. Faber's analysis convinced him that this was a result of the blockade introduced in the preceding year, a restriction that prevented export to take advantage of high prices, forcing the Danes to consume their own not inadequate production. The retention of food and the additional measure of rationing insured a sufficiency for all, so that malnutrition disappeared. To Faber it seemed especially important that a housing shortage did not occur until after the war and that during and after the postwar period of maximum crowding the tuberculosis rate continued to drop. The increase in mortality of the war years was shown by Faber's analysis to be in close relation to the shortage of meat and fish. He concluded that the rise in tuberculosis was specifically the result of protein deficiency.

A difficulty in accepting this view lies in the fact that the drop in the death rate occurred within a few months after the restoration of favorable conditions. Tuberculosis is a chronic disease of slow course, and it seems likely that an unfavorable influence that was in operation for three years would exert its effects for some years thereafter. Other reports, however, suggest that protein deficiency may act rapidly and that the effect may disappear promptly with the return of adequate rations. For example, Høygaard [173] described a sharp rise in the tuberculosis rate and an increase in the severity of the disease in an Eskimo tribe temporarily deprived of meat and fish during a brief famine. It is, of course, possible that other protective factors are concerned.

Studies on Protein, Fat, Carbohydrate and Mineral Nutrition in Relation to Tuberculosis.—Only a few well controlled experimental studies are on record. Lange and Simmonds [174] studied the effect of low, medium and high protein diets on the course of tuberculosis in

172. Faber, K.: Acta tuberc. Scandinav. **12**:287, 1938.
173. Høygaard, A.: Nord. med. tidskr. **16**:1647, 1938.
174. Lange, L. B., and Simmonds, N.: Am. Rev. Tuberc. **7**:49, 1923.

rats. A basic diet of rolled oats, casein, gelatin, dextrin, butter, sodium chloride and calcium carbonate was modified by adding or subtracting casein and gelatin to affect variation in the protein content. Dietary control was commenced when the rats were 6 months old. Ten weeks later they were inoculated subcutaneously with bovine type bacilli, after which sample groups of animals were killed for observation at intervals of one week to five months. At the end of the experiment no differences in the character or in the course of the tuberculosis in the three groups with different protein intakes were apparent.

A similar outcome was observed in a like experiment by Lange [175] on the effect of variable fat nutrition on rat tuberculosis. No influence of low or high fat diet was noted. Levinson [176] studied the effect of fats and lipoids on rabbit tuberculosis. Some gain in weight took place, but a significant protective influence of fats or lipoids was not found.

No controlled experimental studies on the relation of carbohydrates to tuberculosis have been found. Freilich and Coe [177] tested the effect of a high carbohydrate diet on tuberculosis in 35 sanatorium patients and believed that a beneficial effect was demonstrated. This view is at variance with that of a number of epidemiologists who have felt that peoples of low economic level, subsisting largely on carbohydrate, have a nutritional disadvantage with respect to tuberculosis.

Mineral nutrition has been investigated more frequently. A relationship between the healing and the calcification of tuberculous lesions has long been recognized. Early studies on high calcium intake have been succeeded latterly by experiments on the effect of calcification-promoting vitamins. Most of the other reported studies on mineral nutrition have been from clinics.

A large number of articles have appeared in Germany on the Gerson-Sauerbruch-Hermannsdorfer diet, a vitamin-rich and sodium chloride–poor diet containing a proprietary salt mixture, the supposed beneficial effects of which have been much stressed by various authors. The diet is rich in alkaline earths and phosphorus and yields an alkaline ash. The effect on the body is described as "transmineralization." Clausen [168] has reviewed the voluminous literature, in most of which the evidence presented was purely empiric. Impartial evaluation, as he has indicated, fails to demonstrate any theoretic relations to resistance to tuberculosis or any clear evidence of an improvement in resistance resulting from the use of the diet. A few experimental studies, such as those of Blumberg [178] and Bonanno,[179] led to cautious, equivocal statements on the

175. Lange, L. B.: Am. Rev. Tuberc. **11**:241, 1925.
176. Levinson, S. A.: Am. Rev. Tuberc. **23**:527, 1931.
177. Freilich, E. B., and Coe, G. C.: Am. Rev. Tuberc. **34**:293, 1936.
178. Blumberg, W.: Ztschr. f. Tuberk. **59**:305, 1931.
179. Bonanno, A. M.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **77**:19, 1932.

value of the "G-S-H" diet, suggesting that a diet yielding an acid residue is somewhat harmful and one giving an alkaline residue beneficial.

The effect of sodium chloride and calcium carbonate was investigated directly by Lange.[180] Early results led her to believe that deficiency in these salts led to a more diffuse and extensive local reaction to tubercle bacilli than occurs in normally fed animals. Subsequent studies, however, failed to confirm this result, rats on salt-rich and salt-poor diets responding essentially alike to tuberculous infection.

Vitamin A.—Cod liver oil, containing vitamins A and D, has long been considered useful in the treatment of tuberculosis. In early experiments Smith[181] was unable, however, to demonstrate any favorable effect on tuberculosis in guinea pigs, but he reported a curious reduction in tolerance to tuberculin in rats deficient in vitamin A. Hagedorn[182] noted that rats withstood vitamin A deficiency poorly under any circumstances. His article should be consulted for a review of the literature prior to 1929 on the relations of vitamin nutrition and tuberculosis.

An experimental study in mice by Finkelstein[183] indicated that depletion in vitamin A after removal of this vitamin and carotene from the diet resulted in tuberculosis of a more acute course than that in mice receiving 0.005 mg. of carotene daily. The usual evidences of vitamin A deficiency were noted in the depleted animals.

Steiner, Greene and Kramer[184] tested the effect of vitamin A deficiency on tuberculosis in guinea pigs and rabbits. In the former animals the deficiency did not seem to lead to accelerated or more severe tuberculosis as compared with that in controls. It was evident that the guinea pigs fed on any of the artificial diets, whether deficient or rich in vitamins, did not do as well as those on their normal, customary diet. Rabbits on a diet deficient in vitamin A appeared to have slightly more extensive tuberculosis than the controls. However, aversion to the diet may have been as much a factor in the high mortality of the deficient animals as deficiency in the vitamin.

Kon and Maddock[185] fed a small number of swine diets poor and rich in vitamin A. The rich diet did not protect against development of tuberculosis after ingestion of milk from a tuberculous cow.

Studies on man indicate with little doubt that tuberculous persons have much lower quantities of vitamin A in the blood and tissues than

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180. Lange, L. B.: Am. Rev. Tuberc. **15**:629, 1927.

181. Smith, M. I.: Am. Rev. Tuberc. **7**:33, 1923; Pub. Health Rep. **43**:2817, 1928.

182. Hagedorn, K.: Beitr. z. Klin. d. Tuberk. **72**:1, 1929.

183. Finkelstein, M. H.: Proc. Soc. Exper. Biol. & Med. **29**:969, 1932.

184. Steiner, M.; Greene, M. R., and Kramer, B.: Am. Rev. Tuberc. **36**:222, 1937.

185. Kon, S. K., and Maddock, C.: Vet. Rec. **50**:1693, 1938.

healthy persons. Getz, Hildebrand and Finn [186] estimated vitamin A deficiency in patients and control subjects by determining dark adaptation, using the biophotometer method of Jeans, Blanchard and Zentmire. This method indicated a deficiency of vitamin A in 53 per cent of tuberculous patients, contrasting with 6 to 11 per cent of healthy college students. The degree of deficiency was proportionate to the severity of the tuberculosis, and in most instances normal levels could be restored by feeding cod liver oil. Subsequent studies by Getz and Koerner [187] on a large number of tuberculous patients at a clinic, carried out by colorimetric measurement of vitamin A in the blood by the Kimble modification of the method of Dann and Evelyn, have confirmed these results.

A deficiency in the amount of vitamin A in the livers of patients dying from tuberculosis was reported by Crimm and Short.[188] The amounts were especially low in patients who had had a febrile course before death and in those who had suffered from tuberculous enteritis. McConkey [189] reported that the administration of cod liver oil exerted a protective effect against the development of tuberculosis of the intestines.

Vitamin C.—In recent years chief attention in the field of vitamins and tuberculosis has turned to vitamin C. For an analysis of the literature on this vitamin up to 1937, including its relation to resistance to tuberculosis, the reader is referred to the review in the ARCHIVES OF PATHOLOGY by Perla and Marmorston.[190] These authors recognized a favorable effect of vitamin C on resistance and attributed it to the part this vitamin plays in oxidation-reduction processes in cellular metabolism and its importance in the production of intercellular cement substance.

The literature on vitamin C in relation to tuberculosis is large, and a selection will be made of those articles representative of the main trends of investigation. Studies on man and laboratory animals will be described separately.

In the early 1920's several authors correlated scurvy in man and low resistance to tuberculosis (for a review see Perla and Mammorston [190]). Höjer [191] in 1924 described several ways in which the scorbutic state increased susceptibility. Many subsequent reports have indicated that tuberculosis itself depresses the vitamin C content of the blood and tissues. Sweany and his co-workers [192] recently reviewed

186. Getz, H. R.; Hildebrand, G. B., and Finn, M.: J. A. M. A. **112**:1308, 1939.
187. Getz, H. R., and Koerner, T. A.: Unpublished data.
188. Crimm, P. D., and Short, D. M.: Ann Int. Med. **13**:61, 1939.
189. McConkey, M.: Am. Rev. Tuberc. **43**:425, 1941.
190. Perla, D., and Marmorston, J.: Arch. Path. **23**:543 and 683, 1937.
191. Höjer, J. A.: Acta pædiat. **3** (supp.):8, 1924.
192. Sweany, H. C.; Clancy, C. L.; Radford, M. H., and Hunter, V.: J. A. M. A. **116**:469, 1941.

previous studies and presented experience of their own indicating that the severity of tuberculosis is paralleled by "exhaustion" of vitamin C in the body fluids and that a large supplementary intake of vitamin C is necessary to restore the normal levels.

Studies on the urine of tuberculous patients indicate that patients with progressive disease eliminate but small amounts of the vitamin. Heise and Martin [193] found that only 2 per cent of a large group of patients with active tuberculosis excreted more than 14 mg. a day in the urine while living on a sanatorium diet, whereas 39 per cent of the patients with inactive disease and all of the healthy controls studied passed this level of excretion. Difficulty was found in raising the excretion level of patients with active tuberculosis to 14 mg. per day by feeding orange juice, but this level was easily passed in persons without tuberculosis. Bakhsh and Rabbani [194] obtained similar results in a study of 24 patients making poor progress; 19 of them had a low urinary content of vitamin C. Jetter and Bumbalo [195] in an investigation of children confirmed these results; 37 children with active tuberculosis eliminated 5.7 mg. of vitamin C, on the average, per day, whereas the average output of 25 normal children on the same diet was 29.2 mg. The results of Singer and Van Bark [196] in children were similar.

In general, the results from studies of patients indicate that the level of excretion of vitamin C is low in tuberculosis and that this can be correlated with a low content in the blood of the patients. The depletion in reserve in tuberculous patients and the difficulty in raising the blood level to the average of normal subjects indicate that great destruction must take place in tuberculosis. Whether the depletion of itself reduces resistance to the disease, with creation of a vicious circle, is not known, but in view of the apparent susceptibility of scorbutic subjects to tuberculosis, this may be the case.

Numerous studies have been made on the treatment of tuberculosis with excessive quantities of vitamin C. Several investigators have tried to determine whether protection is conferred on nondepleted animals by administration of large amounts of synthetic vitamin C. Heise and Martin [197] maintained a supervitaminosis in guinea pigs for five months by injecting 20 mg. of ascorbic acid daily. The injections were given intraperitoneally, commencing seven days before the infection. In the course of five months each treated animal received 30 Gm. of ascorbic acid. No effect on the course of the tuberculosis was noted, nor, on the

193. Heise, F. H., and Martin, G. J.: Nat. Tuberc. A. Tr. **32**:232, 1936.
194. Bakhsh, I., and Rabbani, M.: Indian M. Gaz. **74**:274, 1939.
195. Jetter, W. W., and Bumbalo, T. S.: Am. J. M. Sc. **195**:362, 1938.
196. Singer, B., and Van Bark, B.: Quart. Bull., Sea View Hosp. **3**:145, 1938.
197. Heise, F. H., and Martin, G. J.: Proc. Soc. Exper. Biol. & Med. **35**:337, 1936.

other hand, was there any deleterious effect from this large amount of vitamin C. Kaplan and Zonnis [198] likewise failed to secure favorable results. On the other hand, Birkhaug [199] claimed that the daily oral administration of 10 mg. of ascorbic acid retarded the progress of tuberculosis, the treated animals showing less caseonecrotic tissue and more fibrosis than nontreated controls. Steinbach, Klein and Duca [200] obtained similar results in guinea pigs. In the rabbit, however, an animal synthesizing its own vitamin C, administration of the synthetic vitamin had no effect on the course of tuberculosis. In this connection may be noted the statement of Osborn and Gear [201] that resistance to tuberculosis in animals has a tendency to run parallel with their capacity to synthesize their own vitamin C.

Controlled comparison of the relation of the scorbutic state and vitamin-rich state to tuberculosis is possible only through animal experiments. Following reports of the favorable influence of orange and tomato juice in preventing tuberculous enteritis in man, McConkey and Smith [202] compared the intestines of tuberculous guinea pigs receiving adequate and inadequate amounts of vitamin C and concluded that an adequate intake was protective against tuberculous ulceration. Greene, Steiner and Kramer [203] found that guinea pigs maintained on a diet deficient in vitamin C had more lesions and ulcers of the intestinal tract than did adequately fed animals. In general, the course of tuberculosis after inoculation of virulent tubercle bacilli was more rapid in the C-deficient animals. However, chronic scurvy did not render the guinea pigs susceptible to a strain of tubercle bacilli of low virulence (R_1).

Thus it seems agreed that scorbutic animals are deficient in resistance, but the evidence for the value of vitamin C in amounts above normal is conflicting.

Vitamin C is believed to decrease capillary fragility and in this way affect capillary permeability. Thus it might affect the reaction of the body to tubercle bacilli or tuberculin. In 1925 Bieling [204] reported that scorbutic guinea pigs were more sensitive to tuberculin than normal ones. Steinbach and Klein [205] in a well controlled experience with

198. Kaplan, A., and Zonnis, M. E.: Am. Rev. Tuberc. **42**:667, 1940.

199. Birkhaug, K. E.: Acta tuberc. Scandinav. **13**:52, 1939.

200. Steinbach, M. M.; Klein, S. J., and Duca, C. J.: Am. Rev. Tuberc. **43**:403 and 415, 1041.

201. Osborn, T. W. B., and Gear, J. H. S.: Nature, London **145**:974, 1940.

202. McConkey, M., and Smith, D. T.: J. Exper. Med. **58**:503, 1933.

203. Greene, M. R.; Steiner, M., and Kramer, B.: Am. Rev. Tuberc. **33**:585, 1936.

204. Bieling, R.: Ztschr. f. Hyg. u. Infektionskr. **104**:518, 1925.

205. Steinbach, M. M., and Klein, S. J.: Proc. Soc. Exper. Biol. & Med. **35**: 151, 1936.

crystalline ascorbic acid obtained similar results and concluded that the daily administration of vitamin C increases the tolerance of tuberculous guinea pigs to large doses of tuberculin. Birkhaug [206] obtained the same result. The degree of inhibition of the tuberculin reaction was closely correlated with the rate of urinary excretion of ascorbic acid and with the concentration of this substance in the adrenal glands as determined by postmortem examination. Heise, Martin and Schwartz [207] reported that in human patients intravenously injected ascorbic acid seemed to lessen sensitivity to tuberculin. They attributed high sensitivity in hypovitaminosis to a greater permeability of the capillaries, which could be corrected by administration of the vitamin.

A number of investigators, however, have found no constant effect of ascorbic acid on the tuberculin reaction. Study of the problem is apparently under way in several places. Raffel and Madison [208] found no significant effect of ascorbic acid on the anaphylactic state in guinea pigs.

A number of authors, including Heise, Martin and Schwartz,[207] have reported that administration of ascorbic acid lowers the sedimentation rate of the tuberculous patient. Birk [209] obtained the same result. The inference drawn was that in tuberculous patients capillary permeability is lowered and that a normal level of ascorbic acid in the blood prevents this state.

Vitamin D.—This vitamin has been less investigated in relation to resistance to tuberculosis than vitamins A and C. It has been shown that calcification of tubercles in animals with experimental tuberculosis can be induced by administration of viosterol (Spies [210]), but it is not certain that resistance to infection and capacity for calcification are related.

If there is a relation between vitamin D and resistance to tuberculosis, rachitic animals should display greater susceptibility, just as do scorbutic animals, deficient in vitamin C. Grant [211] reported in 1927 that the resistance of white rats could be decreased by lowering the calcium and vitamin D of the diet, i. e., by feeding diets that produced rickets. A supplementary study by Grant, Bowen and Stegeman [212]

206. Birkhaug, K. E.: Acta tuberc. Scandinav. **13**:45, 1939.

207. Heise, F. H.; Martin, G. J., and Schwartz, S.: Brit. J. Tuberc. **31**:23, 1937.

208. Raffel, S., and Madison, R. R.: J. Infect. Dis. **63**:71, 1938.

209. Birk, H.: Ueber den Einfluss von Vitamin C auf das Blutbild und die Blutsenkung Tüberkulösen, Thesis, Tübingen, 1939.

210. Spies, T. D.: Am. Rev. Tuberc. **23**:169, 1931.

211. Grant, A. H.: Am. Rev. Tuberc. **16**:628, 1927.

212. Grant, A. H.; Bowen, J. A., and Stegeman, D. E.: Am. Rev. Tuberc. **16**:642, 1927.

indicated that the amount of tuberculosis developing on inoculation of rats depended more on the inherited vitamin reserve than on the severity of the rickets that set in with administration of a vitamin-deficient diet after weaning. Subsequent studies by Grant [213] suggested a complex relationship between vitamin D, vitamin C and available calcium of such character that administration of vitamin D might decrease resistance in states in which the normal balance was disturbed. This lead does not seem to have been followed.

Zeyland and Piasecka-Zeyland [214] found no significant difference in resistance to tuberculosis in two series of guinea pigs, one receiving 25 U. S. P. XI units and the other 750 units of viosterol daily. Steiner, Greene and Kramer [215] studied the effect of vitamin D on tuberculosis in rabbits. Definite rickets was produced by the ration used, but this did not appear to modify the resistance of the animals to either the bovine or the human type of tubercle bacillus or that of animals vaccinated with the human type and inoculated subsequently with virulent bovine type bacilli. The results reported by these two groups of investigators were in accord with the conclusions reached by Clausen [168] from a review of the literature, that no decisive evidence is at hand for an effect of vitamin D on resistance to infection.

COEXISTING DISEASES

General.—A modifying influence of different diseases on tuberculosis has long been accepted as a fact by most investigators. Rokitansky [216] listed a series of diseases as antagonistic. Ickert [217] has recently grouped under the designations "syntropy" and "dystropy," terms coined by Pfaundler, two series of abnormal states, the one favoring and the other opposing the progression of tuberculosis. Each list includes a number of the factors thus far considered in this article and a number of specific diseases as well. He quoted Schulz's [218] investigation of the nontuberculous complications reported as contributory causes of death in cases of tuberculosis, which led to a series of low and high correlations between tuberculosis and certain other diseases. Among the diseases showing high correlation with tuberculosis were diabetes, silicosis, measles, whooping cough and lymphogranulomatosis; among those with low

213. Grant, A. H.: Am. Rev. Tuberc. **21**:102 and 115, 1930.

214. Zeyland, J., and Piasecka-Zeyland, E.: Tubercle **17**:198, 1936.

215. Steiner, M.; Greene, M. R., and Kramer, B.: Am. Rev. Tuberc. **35**:640, 1937.

216. von Rokitansky, C.: Handbuch der speciellen pathologischen Anatomie, Vienna, Braumüller & Seidel, 1844.

217. Ickert, F.: Beitr. z. Klin. d. Tuberk. **94**:383, 1940.

218. Schulz, H.: Reichsgesndhtsbl., 1939, vol. 14, nos. 15, 17, 19 and 21; cited by Ickert.[217]

correlation were scarlet fever, diphtheria, heart and vascular illness, chronic bronchitis and asthma.

Acute Infections.—The most frequent acute complication of tuberculosis is the common cold. Heise [219] found no more relapses in a group of tuberculous patients who had had acute colds than in a group under like conditions who had not suffered from colds. Zoelch [220] found a high association between measles, grip and whooping cough and tuberculosis, and a relatively low association between the latter and scarlet fever, diphtheria and sore throat. The enhancing effect of certain diseases on tuberculosis, particularly measles, was attributed to depression of the state of allergy brought about by the latter, an effect to which attention has been drawn for many years. Various explanations have been given for the depression of the intensity of the tuberculin reaction in measles (Pilcher [221]). There is little disagreement as to the frequent occurrence of such a depression. Other similar diseases have like action, e. g., German measles (Blechman and François [222]).

Gabriel [223] analyzed the relations of whooping cough and tuberculosis in 1,995 cases of the former in Vienna. No evidence was found that whooping cough activated latent or aggravated active tuberculosis. This is contrary to the experience reported by Schulz [218] and indicates the lack of agreement that may occur in studying the problem by different methods. Rumshina [224] found a very low association of tuberculosis and infantile paralysis.

Syphilis.—There has been extensive and continued investigation of the association of tuberculosis and syphilis, and it is still impossible to determine whether the latter affects the course of tuberculosis significantly. The intermediate figure of Schulz [218] on the association did not suggest either a favoring or an antagonistic influence. Only a few of the many recent articles can be cited here; the ones selected indicate some diversity of opinion among clinicians, although it is evident that few authors feel that convincing evidence for an effect of the one on the other is at hand. Schlesinger [225] claimed that syphilis of long standing had little effect on the course of tuberculosis but that recent, active syphilis exerted a harmful influence. No healing effect or tendency to stimulate fibrosis could be attributed, according to his results, to the

219. Heise, F. H.: Am. Rev. Tuberc. **21**:664, 1930.

220. Zoelch, P.: Beitr. z. Klin. d. Tuberk. **93**:549, 1939.

221. Pilcher, J. D.: Am. Rev. Tuberc. **21**:669, 1930.

222. Blechman, G., and François, R. H.: Bull. Soc. de pédiat. de Paris **36**:595, 1938.

223. Gabriel, E.: Jahrb. f. Kinderh. **142**:281, 1934; abstracted, Am. Rev. Tuberc. (Abstr. Sect.) **31**:44, 1935.

224. Rumshina, E.: J. Bone & Joint Surg. **15**:772, 1933.

225. Schlesinger, H.: Beitr. z. Klin. d. Tuberk. **79**:750, 1932.

presence of syphilis. Padget and Moore [226] emphasized the many difficulties in clinical analysis but stated their impression that untreated syphilis affected the course of tuberculosis unfavorably. At the same time they reported a series of cases in which treatment of syphilis appeared to activate previously latent tuberculosis. Warring,[227] after study of a large number of sanatorium patients, stated cautiously that "the course of tuberculosis in the syphilitics seems to be slightly less favorable than in the non-syphilitics." The unfavorable trend was more conspicuous in Negro than in white patients. Goldblatt,[228] in an analysis of the records of 25,472 Negro patients at a clinic, found that tuberculosis had occurred almost twice as frequently among syphilitic as among nonsyphilitic patients.

A study by Guild and Nelson,[229] however, also on an epidemiologic basis, indicated that the effect of syphilis on tuberculosis, if any, could not be serious. In 20,281 tuberculous patients on whom blood tests were made the incidence of syphilis was no higher than in the general population, evidence suggesting that syphilis does not predispose to tuberculosis. Ernst [230] never observed a direct unfavorable influence of syphilis on tuberculosis and found no difference in the life expectancy of tuberculous patients with or without syphilis. In a study of the records of 13,239 adult tuberculous patients Berg [231] found no evidence that the coexistence of syphilis causes tuberculosis to run a more rapid course than it does in the absence of syphilis. No evidence was found to confirm the old view that chronic or latent syphilis favors fibrosis of tuberculous lesions.

Many clinicians have expressed the hope that the mutual action of syphilis and tuberculosis could be investigated in laboratory animals. Aronson and Meranze [232] have made a start on the desired type of experimentation. Their syphilitic rabbits reacted to an intracutaneous injection of living virulent tubercle bacilli in a manner different from that of nonsyphilitic rabbits. The local inflammatory reaction was more intense; multiple focal lesions were evident in three hours and occurred characteristically around the blood vessels, whereas in the nonsyphilitic rabbits the lesions were single, diffuse and not related to the blood vessels. It was noteworthy that the tuberculous lesions in the skin of the syphilitic rabbits displayed a histologic appearance more characteristic of syphilis than of tuberculosis, i. e., an "anamnestic" type of response.

226. Padget, P., and Moore, J. E.: Am. Rev. Tuberc. **33**:10, 1936.
227. Warring, F. C.: Am. Rev. Tuberc. **40**:175, 1939.
228. Goldblatt, S.: Arch. Dermat. & Syph. **40**:687, 1939.
229. Guild, C. St. C., and Nelson, M.: Am. Rev. Tuberc. **33**:31, 1936.
230. Ernst, F.: Med. Klin. **30**:1657, 1934.
231. Berg, S.: Quart. Bull., Sea View Hosp. **4**:402, 1939.
232. Aronson, J. D., and Meranze, D. R.: Am. J. Path. **14**:163, 1938.

No evidence was published indicating that the general course of tuberculosis in the viscera differed in syphilitic and nonsyphilitic rabbits.

Leprosy.—Tuberculosis occurs frequently in patients with leprosy. According to Johansen,[233] "statistics differ for different localities but it is generally stated that about 50 per cent of all deaths in leprosy are attributed to tuberculosis." Whether the relation is due to anything more significant than close contact with persons suffering from open lesions is not known. Certainly the long infection with acid-fast bacilli in leprosy affords no protection against tuberculosis. Pavloff [234] described a case of close association of leprous and tuberculous lesions in the skin.

Diseases of the Hemopoietic System.—Schulz [218] reported that in his series of combined causes of death pernicious anemia and tuberculosis were only rarely associated. Sturgis [235] saw fatal tuberculosis in 1 of 21 necropsies on patients with pernicious anemia. Minor lesions of no clinical significance were seen in 6 of the remainder of these necropsies. Karlmark and Olovson [236] described 2 cases in which the two diseases were combined. In 1 instance the anemia appeared to favor progression of the tuberculosis. Shiskin and Gloyne [237] found that repeated bleeding of rabbits, leading to severe anemia, did not break down the animals' resistance to tuberculosis.

Resistance to tuberculosis appears to be associated with a preponderance of lymphocytes, and susceptibility with lymphopenia, as indicated by blood counts of human patients with lesions of varying progress. Murphy [238] found that irradiation of mice, with resultant reduction of their lymphoid tissue, decreased their survival time after the onset of tuberculous infection as compared with that of nonirradiated controls. For reviews the reader is referred to Sabin,[239] Smithburn [240] and Wiseman and Doan.[241] These investigators found a relatively high lymphocyte count a sign of good resistance and a high monocyte count an index of poor resistance. The monocyte-lymphocyte ratio thus served as a

233. Johansen, F. A.: Am. Rev. Tuberc. **35**:609, 1937.

234. Pavloff, N.: Vestnik venerol. i dermat., 1938, p. 38; abstracted, Zentralbl. f. d. ges. Tuberk.-forsch. **49**:503, 1939.

235. Sturgis, C. C.: Tr. A. Am. Physicians **54**:46, 1939.

236. Karlmark, E., and Olovson, T.: Beitr. z. Klin. d. Tuberk. **79**:772, 1932.

237. Shiskin, C., and Gloyne, S. R.: Am. Rev. Tuberc. **38**:325, 1938.

238. Murphy, J. B.: The Lymphocyte in Resistance to Tissue Grafting, Malignant Disease and Tuberculous Infection: An Experimental Study, Monograph 21, Rockefeller Institute for Medical Research, 1926.

239. Sabin, F. R.: Am. Rev. Tuberc. **25**:153, 1932.

240. Smithburn, K. C.: J. Exper. Med. **56**:193, 1932.

241. Wiseman, B. K., and Doan, C. A.: Am. Rev. Tuberc. **30**:33, 1934.

measure of prognosis. They found that procedures causing a relative decrease in the circulating monocytes increased the survival time of tuberculous animals. However, injection of chicken embryonic extract into rabbits, which increased the number of circulating lymphocytes of immature type, appeared to result in more extensive tuberculous lesions in the lymphatic system. Several other investigators have tried to modify resistance to tuberculosis by methods affecting the tissues forming white blood cells. Corper [242] was unable to reduce the resistance of guinea pigs to tuberculosis by roentgen irradiation, benzene, thorium-x or other substances injuring the body's capacity to generate leukocytes.

Numerous investigators have reported a relation between the spleen and resistance (see Perla and Marmorston [62] for citations), but their claims are contradictory. Lewis and Margot [243] found that removal of the spleen increased the resistance in mice. Foot [244] discovered in rabbits, after splenectomy a changed distribution of the tuberculous lesions, rather than a reduction in number. Deinse [245] could detect no change in resistance in laboratory animals after splenectomy. Marmorston [246] reported that removal of the spleen in mice diminished resistance to human and bovine type tubercle bacilli, a result quite at variance with that reported by Lewis and Margot.[243]

Several reports have been made on the association of tuberculosis with various types of leukemia and Hodgkin's disease. Parker and his associates [247] found active tuberculosis in 6 of 30 cases of the latter disease and noted that an association had been recognized ever since Sternberg called attention to the frequent concomitance of the two diseases, in 1898. In contrast, Parker and his associates found no active tuberculosis in cases of lymphosarcoma, lymphocytoma, reticulum cell sarcoma, myeloma or myeloid leukemia. An occasional association of active tuberculosis with lymphatic leukemia was found, and healed tuberculosis was not infrequently discovered in the presence of all of the conditions named and also in that of pernicious anemia. For other reports on the association of leukemia and tuberculosis reference may be made to Holler,[248] Jaffé [249] and Ryan and Medlar.[250] Jaffé described

242. Corper, H. J.: Am. Rev. Tuberc. **2**:587, 1918-1919.
243. Lewis, P. A., and Margot, A. G.: J. Exper. Med. **19**:187, 1914.
244. Foot, N. C.: J. Exper. Med. **38**:263, 1923.
245. Deinse, F.: Compt. rend. Soc. de biol. **124**:893, 1937.
246. Marmorston, J.: Am. Rev. Tuberc. **36**:119, 1937.
247. Parker, F.; Jackson, H., Jr.; Bethea, J. M., and Otis, F.: Am. J. M. Sc. **184**:694, 1932.
248. Holler, G.: Klin. Wchnschr. **10**:1663, 1931.
249. Jaffé, R. H.: Am. Rev. Tuberc. **27**:32, 1933.
250. Ryan, W. J., and Medlar, E. M.: Am. Rev. Tuberc. **36**:212, 1937.

3 cases of myeloid leukemia in which the leukemia appeared responsible for reactivation of tuberculosis and noted that the leukemic cells did not take part in the defense reaction. Ryan and Medlar confirmed the traditional view that a high count of lymphocytes normally indicated a good prognosis, but in a case of combined tuberculosis and lymphatic leukemia in their own experience the leukemia had no evident influence on the tuberculosis.

Malaria.—The coincidence of tuberculosis and malaria has been described frequently. There is no unanimity of opinion as to their mutual effects. In Italy Magliano [251] believed that malaria of long standing favored fibrosis in cases of pulmonary tuberculosis and that malaria of recent date had no effect. Garofali [252] believed a favorable influence was exerted by malaria at any time, because of stimulation of the reticuloendothelial system. Torossjan [253] likewise reported a beneficial effect of malaria. On the other hand, an epidemiologic survey by Gioseffi [254] in a malarial region disclosed tuberculosis in numerous persons, acute in many of them. A survey by Kasieff [255] in Baku showed frequent association of the two diseases. In his experience acute malaria always had an unfavorable influence on tuberculosis, while chronic malaria was without effect.

Shirokogorov [256] found primary foci of tuberculosis in about a quarter of necropsies on patients dying from acute or chronic malaria. The foci and neighboring tissue gave no evidence of reactivation by the malaria, even in cases of profound malarial anemia with cachexia, amyloid degeneration and other secondary changes. Active tuberculosis was found in only 20 of the 800 necropsies. Shirokogorov concluded that malaria could not be considered an activator of tuberculosis.

A brief review on the relations of tuberculosis and tropical diseases was written by Flinn,[257] who believed that malaria, hookworm, ascaris infection and other diseases common in the tropics increased the severity of tuberculosis. On the whole, however, the evidence from many sources is so contradictory that it seems doubtful if malaria has much effect on the progress of the disease.

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251. Magliano, E.: Ann. Inst. Maragliano **7**:157, 1937.

252. Garofali, F.: Tubercolosi **24**:335, 1932.

253. Torossjan, G.: Bor'ba s. Tuberk., 1935, no. 1, p. 79; abstracted, Zentralbl. f. d. ges. Tuberk.-forsch. **44**:359, 1936.

254. Gioseffi, M.: Riforma med. **54**:1548, 1938.

255. Kasieff, Y. M.: Probl. tuberk., 1938, no. 6, p. 60; abstracted, Zentralbl. f. d. ges. Tuberk-forsch. **50**:731, 1939.

256. Shirokogorov, I. I.: Klin. Med. **12**:1786, 1934.

257. Flinn, Z. M.: Puerto Rico Health Bull. **4**:111, 1940.

Icterus.—Trautwein [258] reported 2 cases in which previously regressing tuberculous lesions appeared to be reactivated by catarrhal jaundice. He referred the unfavorable effect to injury of the reticuloendothelial system by bile in the circulation.

Cancer.—The mutual effects of cancer and tuberculosis have been a favorite subject of investigation. A statistical study by Pearl [259] in 1929 stimulated much research. He reported active tuberculous lesions in 16.3 per cent of 816 persons without cancer and in only 6.6 per cent of a corresponding number of patients with malignant growths. When the figures were further analyzed, he found that in every decade and in each sex and race division the percentage of persons with active tuberculous lesions was higher in the noncancerous than in the cancerous group. A study from the reverse point of view on 886 tuberculous patients showed the same relationship. He concluded that a distinct antagonism exists between cancer and tuberculosis.

Carlson and Bell [260] agreed after an analysis of the records of tuberculosis and cancer in 11,195 necropsies that active tuberculosis is less frequent in cancerous than in noncancerous subjects and that cancer is less common in those with active tuberculous lesions than in those without active tuberculosis. They maintained, however, that this failure of association of the two diseases did not mean that cancer and tuberculosis are antagonistic. Heart disease showed just as little association with tuberculosis. They believed the apparent antagonism could be explained on the ground that the majority of persons with active tuberculosis have no other disease, and therefore noncancerous persons would always show a higher percentage. In their opinion the only proper control in a study of the association of active tuberculosis and cancer is the incidence of active tuberculosis in cases of some other disease.

Moore and Schmeisser [261] studied the combined incidence of the two diseases in 2,000 necropsies. Approximately 4 per cent of all persons with tuberculosis had malignant tumors, and 16 per cent of all persons with tumors had tuberculosis. They noted definite relations with age, sex and race. Simultaneous occurrence was not rare. It occurred most frequently (2.73 per cent) in white women more than 35 years old. In general, the incidence of combined lesions appeared to correspond with what was expected on the basis of the frequency with which the two diseases were observed independently in the different sexes at different ages.

Lenti [262] described 28 cases of simultaneous tuberculosis and carcinoma of the lung. He expressed the belief that no antagonism existed.

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258. Trautwein, H.: Beitr. z. Klin. d. Tuberk. **93**:594, 1939.
259. Pearl, R.: Am. J. Hyg. **9**:97, 1929.
260. Carlson, H. A., and Bell, E. T.: J. Cancer Research **13**:126, 1929.
261. Moore, R. M., and Schmeisser, H. C.: Am. Rev. Tuberc. **35**:336, 1937.
262. Lenti, P.: Arch. ital. di anat. e d'histol. pat., 1936, supp., p. 171.

Pulmonary tuberculosis seemed capable of acting as a chronic irritant stimulating the growth of cancer, and cancerous cachexia, on the other hand, appeared to activate tuberculosis with the production of more acute lesions.

Teutschländer [263] investigated the mutual effects of tuberculosis and the Rous sarcoma experimentally in chickens. An apparent antagonism existed in that progression of tuberculosis caused regression of the Rous tumor. However, the tumor had no effect on the course of the tuberculosis. In the opinion of the author the effect of tuberculosis in bringing about regression of the Rous sarcoma was nonspecific.

Circulatory Disturbance.—Rokitansky [216] considered venosity and cyanosis of paramount significance in the inhibition of tuberculous lesions. In his belief any lesion of the heart increasing the venous congestion of the lungs had a suppressing influence on tuberculosis. Subsequently an antagonism between tuberculosis and mitral stenosis was generally recognized. Gloyne and Shiskin [264] found pulmonary tuberculosis in only 1 of 121 cases of mitral stenosis. Of 45 cases of sclerosis of the mitral valve without the characteristic mitral stenosis, tuberculosis was found in 3. The authors concluded that a true antagonism between tuberculosis and mitral stenosis existed, which might have been the result of immunity due to excess of blood in the lung, pulmonary fibrosis from long-continued congestion or an alteration of the hemorespiratory exchange, with a less alkaline p_H of the blood. Such results probably represent the usual experience. However, not infrequently cases are reported in which pulmonary tuberculosis is progressive in the presence of severe mitral stenosis (for example, Patoir and Watel [265]).

Gloyne [266] searched 2,735 autopsy records for cases of combined congenital heart disease and tuberculosis. A number were discovered, but not enough for valid conclusions as to the interrelations of the two conditions. One case of tuberculosis with congenital stenosis of the pulmonary artery appeared significant for the view that defective blood supply may predispose to tuberculosis. In this connection may be noted Pagel's [267] observation of unilaterally massive tuberculosis in a case of marked stenosing endarteritis of one pulmonary artery.

Hyndman and Landt [268] studied the influence of passive congestion on tuberculosis by observing the progress of this disease in the abdominal organs after complete or partial ligation of the vena cava. The progress

263. Teutschländer, O.: Ztschr. f. Krebsforsch. **30**:498, 1930.
264. Gloyne, S. R., and Shiskin, C.: Tubercle **18**:394, 1937.
265. Patoir, A., and Watel, E.: Arch. méd.-chir. de l'app. respir. **13**:451, 1938.
266. Gloyne, S. R.: Tubercle **17**:455, 1936.
267. Pagel, W.: Brit. M. J. **1**:922, 1935.
268. Hyndman, O. R., and Landt, H.: Arch. Surg. **28**:684, 1934.

of individual lesions was not checked, but congestion appeared definitely to reduce the number of lesions.

Hypertension.—Most observers have found tuberculosis infrequent in cases of hypertension. Ayman [269] reviewed the literature on the rarity of association of the two conditions and reported his own experience with 240 cases of active tuberculosis. In only a single instance was the arteriolar pressure above 150 mm. The infrequency of tuberculosis in cases of hypertension did not seem explainable on the basis of age or household contact with tuberculous patients, but the study failed to reveal the reason for the rarity of association.

Similar experiences could be cited, such as that of Bunta,[270] who found absolute hypertension in only 14 of 1,356 tuberculous patients, and Masten,[271] who found it in 2.6 per cent of 230 patients. In Masten's experience most tuberculous patients who improved had either stationary or rising arterial blood pressure. This and other experiences suggest that one reason for the rarity of association of hypertension and pulmonary tuberculosis is in the fact that the latter lowers blood pressure. Significant results will be available only when large numbers of persons with hypertension who are known to have had contact with tuberculous persons can be followed, with observation of the character of tuberculosis if it develops.

Silicosis.—Of all supposed instances of reduction of resistance to tuberculosis by independent disease there seems to be least argument about those in which the reduction is ascribed to silicosis. Hence no attempt will be made to abstract the abundant literature on this subject. There is a growing tendency to stress the chronicity of tuberculosis associated with silicosis, but the initially predisposing influence of silicosis does not seem to be denied. The difficulties dependent on such factors as environment and dosage in the evaluation of human disease have been avoided in the experimental studies. Among the most significant of these were Gardner's [272] early observations that a strain of tubercle bacilli of very low virulence. (R_1) caused progressive disease in the lungs of guinea pigs exposed to silica dust, that such lesions ran a much more prolonged course than did the lesions produced in nondusted animals, which tended to heal, and finally, unlike what happened in nondusted animals, the spread of the tuberculous process to the regional lymph nodes was not prevented in dusted animals. A review by Gardner [273] may be consulted for results of subsequent research. In the intervening

269. Ayman, D.: Am. J. M. Sc. **178**:712, 1934.
270. Bunta, E.: Am. Rev. Tuberc. **29**:335, 1934.
271. Masten, A. R.: Am. Rev. Tuberc. **27**:342, 1933.
272. Gardner, L. U.: Am. Rev. Tuberc. **4**:734, 1920.
273. Gardner, L. U.: Am. Rev. Tuberc. **29**:1, 1934.

years a mutual effect of silicosis and other infections was discovered. No satisfactory explanation of the effect of silicosis on tuberculosis has been reached. The action appears to be on the tissue, not on the bacillus. Vorwald and Delahant [274] concluded from a series of experiments in guinea pigs and rats that silica possibly altered the monocytic cells in such a way as to favor growth of tubercle bacilli. Their experiments showed that not only native but acquired resistance was depressed by silica.

Alcohol.—It is traditional that persons with alcoholism have impaired resistance to tuberculosis, but satisfactory evidence is not available. Brown and Eagan [275] have reviewed the literature on the subject. A few decades ago alcohol was used extensively in the treatment of tuberculosis. Orth [276] believed alcohol to be a factor contributing to the progression of tuberculosis only so far as it led to poor housing and nutrition, i. e., indirectly through the production of "alcohol pauperism." Orth carried out a small number of experiments on rabbits, in which no great differences were found in the course of tuberculosis in alcoholized and control animals. Long [277] found no definite differences in the progression of tuberculosis in mice on a normal diet and mice in a state of frequent and protracted intoxication from alcohol in their drinking water.

SUMMARY AND CONCLUSIONS

Studies on constitution in the old sense of body type have not been fruitful for the understanding of resistance to tuberculosis. There is no proof from an abundant literature that skeletal structure, the "asthenic" build or the character of the integument is concerned in the course of tuberculosis. There is some evidence that a physiologically distinct type of body with high capacity for inflammatory response to injury ("exudative diathesis") is associated with good resistance to tuberculosis, but the claims are not supported by evidence with the degree of control considered necessary in the experimental laboratory.

Failure to take sufficient account of the influence of environment, particularly of variation in the degree of exposure to tuberculosis, has made many elaborate studies on the relation of the state of the body to resistance to tuberculosis of doubtful significance. This is true of a number of meticulous studies, impressive in their accumulation of detail, on the course of tuberculosis in long family lines. Those investigators who have carried out long studies on resistance in man have been fully aware

274. Vorwald, A. J., and Delahant, A. B.: Am. Rev. Tuberc. **38**:347, 1938.
275. Brown, L., and Eagan, J. T.: Am. Rev. Tuberc. **27**:217, 1933.
276. Orth, J., cited by Brown and Eagan.[275]
277. Long, E. R.: Unpublished data.

of the necessity of ruling out environmental differences before estimating constitutional resistance, but the difficulties are often insurmountable.

However, there are studies appearing to satisfy the rigid requirements of proper control which indicate that hereditary differences in resistance to tuberculosis occur in both man and animals. Long-continued observations by several investigators on the course of tuberculosis in identical and nonidentical twins indicate a much greater degree of conformity in response to tuberculosis in the former than in the latter regardless of environment. The evidence suggests hereditary similarity in reaction, but it cannot be denied that the psychic element as represented by similar behavior in identical twins may have an effect. However, laboratory experiments on resistance to tuberculosis in which litter mates from parents of highly inbred stock were compared are on record, which are much less subject to objection. Such studies demonstrate much variation in resistance in different families of a single species, as the rabbit, transmissible through generation after generation by brother and sister mating. Families of high and low resistance can be maintained, differing from each other in the chronicity of course of tuberculosis and the anatomic character of the disease. A significant fact is that stocks of low native resistance are inferior in their capacity to acquire resistance.

Selection of stock resistant to tuberculosis through the operation of the principle of survival of the most fit has not yet been effected in laboratory experiments, but since stocks of high resistance are known to exist, analogy with the selection that occurs in the survival of mice after artificial epidemics of other infectious disease suggests that selection for resistance to tuberculosis can be accomplished.

Investigations on the role of race in resistance to tuberculosis have led to continuing argument. There is no question that the Negro population has a much greater mortality from tuberculosis than the white. Yet the Negro mortality rate today is no higher than the white mortality rate thirty-five years ago. If the high Negro mortality is evidence of a constitutional lack of resistance now, it seems fair to attribute the identical white mortality of 1905 to similar innate susceptibility. It seems much more likely that the difference in mortality rate in Negroes is in large part a result of difference in environment, and it has been proved that both races have responded to improving environment with a continuously dropping death rate.

However, certain differences in the anatomic character of tuberculosis in the two races, particularly as exemplified by the tendency toward exudative lesions and massive caseation of lymph nodes in Negroes, suggest that there are some true hereditary differences between the two races. It is true also that highly chronic disease is not infrequent in Negroes. The facts suggest that Negroes, like white persons, are a

mixed group susceptible to the influence of natural selection and that this principle has had a shorter and therefore less effective period of operation in their race. Numerous instances of rapid elimination of the majority of a primitive population by an intense epidemic of acute tuberculosis with ultimate survival of a smaller population displaying a chronic type of tuberculosis are on record.

Age and sex are concerned in resistance to tuberculosis. Age itself probably selects in a heavily infected population through the early removal of susceptible stock. However, variations in resistance due to physiologic differences seem to exist. In spite of exposure children in the decade from 4 to 14 years relatively rarely acquire tuberculosis, and adolescent girls, apparently in spite of less frequent exposure, as indicated by tuberculin tests, break down with tuberculosis more frequently than boys of the same age. Varying periods of exposure seem necessary at different ages to effect breakdown, and the course of fatal disease is more prolonged in the late than in the early periods of life.

Empiric evidence, particularly that derived from epidemiologic experience in times of famine, suggests that malnutrition reduces resistance to tuberculosis. The view has not been well substantiated by controlled laboratory experiments. Analysis of postwar records after 1918 implicated protein deficiency as largely responsible for the high incidence of tuberculosis in the late years of the World War. Confirming laboratory evidence has not been obtained. It is clear that great depletion of the body's stores of vitamins A and C occurs in tuberculosis and the depletion may in turn further reduce resistance. Scorbutic animals appear of greater susceptibility than normal ones.

The constitutional state of the body and its organs may be modified by disease. Diabetes, silicosis and probably lymphogranulomatosis are associated with reduced resistance to tuberculosis. Leprosy may increase susceptibility, and those diseases that reduce allergy, particularly measles, may depress resistance temporarily. Hyperthyroidism, mitral stenosis with pulmonary congestion, and arteriolar hypertension appear associated with increased resistance. The underlying mechanisms affecting resistance favorably or unfavorably are not known. There is no strong evidence that syphilis, malaria, leukemia and cancer affect resistance to tuberculosis.

Notes and News

Appointments.—Edmund V. Cowdry, professor of cytology in Washington University School of Medicine, St. Louis, has been appointed professor of anatomy in that school to succeed Robert J. Terry, retired.

Hardy A. Kemp, dean and professor of bacteriology and preventive medicine in the University of Vermont College of Medicine, has been appointed medical dean of Ohio State University, to succeed J. H. J. Upham.

Awards.—The College of Physicians of Philadelphia has awarded the Alvarenga Prize to John J. Bittner, of the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, "in recognition of his distinguished studies on cancer."

Deaths.—Meyer Bodansky, director of the John Sealy Memorial Laboratory and professor of pathological chemistry, University of Texas School of Medicine, Galveston, died on June 14, 44 years old.

Crime Detection Laboratory.—According to *Northwest Medicine*, a crime detection laboratory has been established in the Tacoma General Hospital under the direction of Charles P. Larson, the pathologist of the hospital. The services of the laboratory will be available to the law-enforcing agencies throughout the state of Washington.

Society News.—The officers of the American Society of Clinical Pathologists for 1941-1942 are: president, J. L. Lattimore; president-elect, Harry Goldblatt; vice president, Russell Hayden; secretary-treasurer, A. S. Giordano.

Book Reviews

The Endocrine Function of Iodine. William Thomas Salter, assistant professor of medicine, Harvard Medical School, and associate physician, Thorndike Memorial Laboratory, Boston City Hospital. Pp. 351. Price $3.50. Cambridge, Mass.: Harvard University Press, 1940.

This volume is announced as the first of a series of "Harvard University Monographs in Medicine and Public Health," to be published under the editorial supervision of Walter B. Cannon, A. Baird Hastings, James Howard Means and S. Burt Wolbach. Such distinguished sponsorship and direction assure honesty of purpose and workmanship, irrespective of the degree to which the individual author may attain the goal he sets himself.

The subject matter, as indicated in the chapter headings, includes: iodine balance and endocrine balance; iodine stores in body tissues; iodine compounds of biologic importance; circulating iodine; thyroid activity; endocrine balance; iodine and the pituitary-ovarian axis; neurologic influence; iodine balance; radioactive iodine; clinical problems. The book concludes with an appendix for laboratory workers. The author, who has himself contributed much important research on some of these topics, ably summarizes the present state of knowledge in this field. The knowledge is considerably greater than it was a few years ago, and much of the information is presented here for the first time in an organized form which makes it generally available to medical scientists and practitioners. It is therefore no reflection on the author or his book to say that he raises many more questions than he is able to answer.

This book will serve as a most useful reference work to all serious students of thyroid function and disorders, whether in the laboratory or in the clinic. Although the average physician will find much of interest but little of immediate practical applicability, no internist who presumes to speak with authority on thyroid problems can afford to be unfamiliar with the contents of this publication.

Manual of Clinical Chemistry. Miriam Reiner, M.Sc., assistant chemist, Mount Sinai Hospital, New York. Pp. 295, with 18 illustrations. Price, $3. New York: Interscience Publishers, Inc., 1941.

Reiner's "Manual of Clinical Chemistry" presents in one compact volume a compendium of methods for a large number of determinations required of the hospital chemical laboratory. This book thus makes unnecessary the usual hunt through numerous textbooks on physiologic chemistry or the consultation of a multitude of original articles in order to find methods applicable to a problem in hand.

The manual contains just enough theoretic explanation of the methods to make execution intelligent; the explanatory matters are stated concisely and do not obscure the practical details. With each method, the range of normal values is given, and there are several excellent tables of the abnormal findings in disease.

Not only are methods for the usual blood, urine and cerebrospinal fluid determinations to be found in the manual, but also methods for those determinations necessitated by the more recent chemotherapy, for example, that employing sulfanilamide. The more frequently used toxicologic tests, as well as tests for vitamins and hormones, and tests for kidney and liver function, are also outlined.

The author wisely includes methods for the preparation of substances which the hospital chemist is often called on to deliver, such as dextrose for intravenous use and certain antigens.

The methods chosen coincide in general with those in use for many years in the reviewer's laboratory, where they have been found to be both accurate and

practical. (In this connection it may be suggested that the Schoenheimer-Sperry method for the determination of cholesterol and its esters be included in the next edition.) The author is to be complimented on having done a remarkably good piece of work in covering so much ground in so concise and handy a volume.

The Periodicity and Cause of Cancer, Leukaemia and Allied Tumours, with Chapters on Their Treatment. J. H. Douglas Webster, M.D., F.F.C.P.E., F.F.R., honorary director, Meyerstein Institute of Radiotherapy, Middlesex Hospital, London, etc. Pp. 178, with plates and charts. Price $3.50. Baltimore: Williams & Wilkins Company, 1940.

After perusing this work, the reviewer has the impression that the chief interest of the author, an eminent British radiologist, is to secure evidence in favor of the virus theory of cancer. The frontispiece depicts a nipple involved in Paget's disease in which the Paget cells are discharging the supposed virus, in the form of fine granules, into the mammary duct. Other plates show elementary bodies in leukemia cells and the historic forms of cancer cell inclusions in several forms of cancer. In some plates the virus is shown leaving the cell, while in others the virus is shown entering the cell.

The well known periodic variations in the growth of malignant tumors are interpreted by Dr. Webster as signifying the cyclic variations in the growth of the virus. The period of thirty-three weeks is stated to be the most frequent period between growth peaks, but half peaks and other variations are observed, so that the general conclusion is that "recurrences appeared at wholly irregular intervals." Thus, in a case of cerebellar angiosarcoma the peaks came at intervals of three, four and eleven months. In a series of cases of breast tumor observed four to twenty-four years, nearly every possible interval was recorded.

The observations have not been controlled by references to grades of malignancy, but the author promises to do this later. He states that the records have been discussed with a competent statistician, but there is no report of the comments of the statistician. The dates of renewed growth seem to have been determined by the visits of the patient or by the appearance of obvious lumps. The general nutrition of the patients is not mentioned. In some cases in which there was prolonged observation the periods gradually became shorter. All forms of cancer as well as of leukemia and Hodgkin's disease are accepted as suitable material for the study. Sixty successful predictions of recurrence are recorded, but not the failures. It is suggested that the period when metastases occur after trauma depends on the periodicity stage of the primary tumor when the trauma occurred.

In the first chapters the author maintains a somewhat serious attitude toward his thesis, endeavoring to show a notable coincidence between recurrences of disease and the prescribed periods. In part II the author seems to abandon his original ambition and admits the occurrence of many variable periods, and finally he becomes deeply submerged in the complexities of the virus theory. Part III includes a discussion of various constitutional remedies for cancer.

The natural growth rhythms in certain malignant tumors are well known to clinicians, especially as concerns cases of prolonged disease. Similar rhythms have been noted repeatedly in normal growth. Why they occur in cancer is not well understood, and careful investigations of this subject are not available. With such a formidable task before him, the author would perhaps have done better had he confined his efforts to the main topic instead of entangling it with another highly controversial phase of the causation of cancer. The chief value of the work is in the renewal of attention to certain clinical peculiarities in the course of cancer.

Books Received

ESSENTIALS OF ENDOCRINOLOGY. Arthur Grollman, Ph.D., M.D., associate professor of pharmacology and experimental therapeutics in Johns Hopkins University. Pp. 480, with 74 illustrations. Price $6. Philadelphia: J. B. Lippincott Company, 1941.

COLLECTED PAPERS OF THE MAYO CLINIC AND THE MAYO FOUNDATION. Edited by Richard M. Hewitt, B.A., M.A., M.D.; A. B. Nevling, M.D.; Harry L. Day, Ph.B., M.D.; John R. Miner, B.A., Sc.D.; James R. Eckman, A.B., and M. Katherine Smith, B.A. Volume XXXII, 1940, published in May 1941. Pp. 1190, with 210 figures. Philadelphia: W. B. Saunders Company, 1941.

THE AMERICAN ILLUSTRATED MEDICAL DICTIONARY: A COMPLETE DICTIONARY OF THE TERMS USED IN MEDICINE, SURGERY, DENTISTRY, PHARMACY, CHEMISTRY, NURSING, VETERINARY SCIENCE, BIOLOGY, MEDICAL BIOGRAPHY, ETC., WITH THE PRONUNCIATION, DERIVATION, AND DEFINITION. W. A. Newman Dorland, A.M., M.D., F.A.C.S., Lieut.-Colonel, M.R.C., U. S. Army; Member of the Committee on Nomenclature and Classification of Diseases of the American Medical Association; editor of the "American Pocket Medical Dictionary." With the collaboration of E. C. L. Miller, M.D., Medical College of Virginia. Nineteenth edition, revised and enlarged. One thousand, six hundred and forty-seven pages, with 914 illustrations, including 269 portraits. Flexible and stiff binding. Plain, $7. Thumb-indexed, $7.50. Philadelphia and London: W. B. Saunders Company, 1941.

COMMUNITY ORGANIZATION FOR HEALTH EDUCATION. A committee report presented by the Committee on Community Organization for Health Education of the American Public Health Association to the Public Health Education Section and the Health Officers Section. Pp. 121. Cambridge, Mass.: The Technology Press, 1941.

INFANTILE PARALYSIS: A SYMPOSIUM DELIVERED AT VANDERBILT UNIVERSITY, APRIL, 1941. Published by the National Foundation for Infantile Paralysis, Inc., 120 Broadway, New York City. Pp. 239. Price $1.25. Baltimore: Waverly Press, 1941.